Hierarchical Modelling In Herpetology: Applications In Ecological And Conservation Studies

Andrea Costa

University of Genova, 2020 - PhD Thesis

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UNIVERSITÀ DEGLI STUDI
DI GENOVA

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PhD program in Science and Technologies for the Environment and the Landscape (STAT)

XXXII cycle in Environmental Biology

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“Hierarchical modelling in herpetology: applications in ecological and conservation studies” (2020).

External Reviewers: Prof. Richard Griffiths, from the University of Kent, and Dr. Giacomo Tavecchia, from the University of the Balearic Islands.

Cover: Speleomantes strinatii (Aellen, 1958) on a stream bank. Photo by Andrea Costa.

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Thesis overview

1. Introduction

Despite the debate about the definition and subject of ecology, it is actually undeniable that the scientific study of the distribution and abundance of organisms, together with their underlying processes, is among the main subjects of ecology (Andrewartha and Birch, 1954; Krebs, 1972; Schwarz and Jax, 2011). Therefore, counting animals, and understanding the mechanisms that drive occurrence and abundance over space and time, have been the focus of many studies in animal science. Since population size is one of the fundamental state variables in ecology, obtaining accurate information on population abundance is of primary importance for conservation, monitoring and management of animal populations. Moreover, accurate population estimates allow the analysis of the temporal variation of abundance (i.e. trends detection), which is a major objective in species conservation and monitoring programs (Seber, 1982; Yoccoz et al. 2001, Williams et al. 2002). Population abundance, however, is not the only candidate variable for ecological and conservation studies: other variables may be used to infer the status of a community or a population. For example, the proportion of area occupied by a species, i.e. Occupancy, is another important state variable of ecology. Although the use of Occupancy, instead of abundance, may appear limiting for drawing inferences about the status of a population or a species, Occupancy is actually related to temporal stability of populations (Glazier, 1986). Occupancy also reflects habitat choice (or resource selection) and a higher Occupancy is usually linked to higher abundances (Gotelli and Simberloff, 1987; Gaston, 1996; Holt et al., 2002). No matter what state variable we decide to adopt, the inference about abundance and Occupancy must face a critical aspect: these two variables, indeed, cannot be directly measured, because species occurrence and population abundance are always subject to imperfect detection. Usually, only a fraction of the population of interest is encountered and available for sampling, while an unknown fraction is unavailable and undetected (Thompson et al., 1998; Williams et al., 2002). This issue led ecologists to the development of several techniques, for taking into account imperfect detection, and obtaining unbiased estimates of population abundance. Several methods rely on individual identification, such as capture-mark-recapture (CMR) and removal sampling, other methods take into account auxiliary data, such as distance sampling (White et al., 1982; Williams et al., 2002; Buckland et al., 2015). These methods are widely capable of overcoming the issue of imperfect detection: however, they may be expensive in terms of costs and efforts, they can be applied to few populations at a time

and they are not practical for large scale monitoring. In the last two decades, the development and application of Hierarchical Models (HMs) in ecology contributed to overcome this issue. HMs, from a mathematical point of view, are a sequence of probability models, ordered by their conditional probability structure, that describe conditionally dependent random variables (Kèry and Royle, 2016). In other terms, and for what concerns the ecological applications in the present thesis, HM describe both the state variable (which is not directly observable), and the measurement error in the observation process. HMs are indeed composed by a sub-model for the true state of interest, that may be Occupancy or abundance, and a sub-model for the observation process, which allows to estimate detection probability. From the application of HMs in ecology, two main tools emerged: Occupancy models (MacKenzie et al., 2002), and N-mixture models (Royle, 2004). From these foundational works, many other models have been developed, but at a bottom level HMs in ecology share some common principles and characteristics: i) they usually require a spatially structured design (several sampling locations), ii) they rely on repeated surveys over sampling locations (sampling sites should be visited several times), iii) they do not require individual identification, since they use presence/pseudo-absence or count data, and iv) the ecoogical and the detection process can be modelled as a function of environmental or sampling covariates (Kèry and Royle, 2016). These characteristics make HMs a useful framework for scientific studies in ecology and also for conservation and management applications. The meta-population design of HMs indeed allows to obtain estimates of the state variable for several populations, or wide study areas, at once. Abundance (but also other demographic parameters such as recruitment or survival), Occupancy and detection probability can be inferred on the basis of environmental variables or management options, trough the inclusion of appropriate covariates in the modelling process. Last but not least, the cost-effectiveness of HMs: the use of cheap methods to collect data, such as simple counts and presence/psesudo-absence data, make these methods highly recommended when time and economic resources are limited. Indeed, the application of HMs seems to be increasing over time, since their first formulation by MacKenzie et al. (2002) and Royle (2004) (Figure 1), and they received great interest by both scientists and managers in several fields of environmental science (Figures 2). The aim of the present thesis is to evaluate the application of HMs in the ecology and conservation of Amphibians and Reptiles.

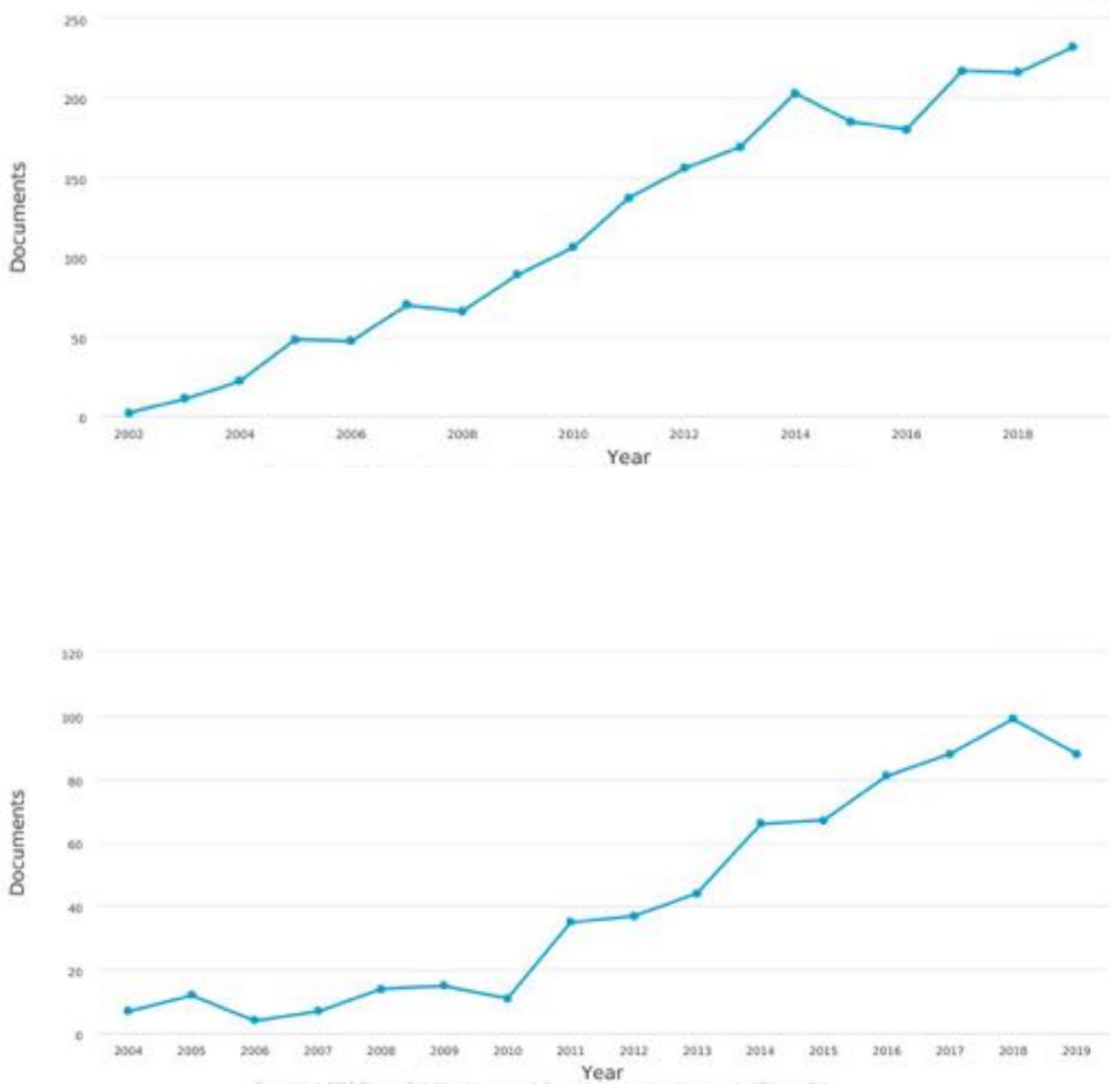

Figure 1 Citation trend of MacKenzie et al.'s (2002 - top) and Royle's (2004 - bottom) foundational works, respectively on Occupancy and N-mixture models. Source of the data: Scopus, December 2019.

Figure 2 Word-cloud composed by the keyowrds of the articles that cite MacKenzie et al.'s (2002 - top) and Royle's (2004 - bottom) foundational works, respectively on Occupancy and N-mixture models. Font size represent the frequency of each keyword. Source of the data: Scopus, December 2019.

Amphibians and reptiles, indeed, are experiencing a dramatic decline worldwide: habitat loss and alteration, alien species and pathogens being the main drivers (Longcore et al., 1999; Gibbons et al., 2000; Stuart et al., 2004; Martel et al., 2013). Therefore, obtaining reliable information on the ecology and abundance of amphibian and reptile population is pivotal in order to address proper conservation strategies. Because of that, these vertebrates recently received great and increasing attention. In particular, newly developed tools in ecology, accounting for imperfect detection, are encouraged and often applied to amphibians and reptiles (Schmidt, 2003; Mazerolle et al., 2007; O'Donnell and Semlitsch, 2015; Griffiths et al., 2015; Mazerolle, 2015). HMs, from their first appearance, have been widely employed in herpetological studies (Figure 3), with an increasing and wide range of application (Figure 4). In the present thesis I employed HMs on several species and with different aims: for example, I adopted HMs in order to understand the fine scale habitat selection of a forest-dwelling salamander (Chapters 1) or to drive conservation plans for salamanders in managed forests (Chapters 2 and 3). Moreover, I tested the reliability of count data for a long-term monitoring on a Gecko population experiencing e strong decline (Chapter 6 and 7). Finally, I compared the results of traditional CMR methods, with those obtained from different N-mixture protocols (Chapter 5). The application of HMs , under different conditions and different Taxa, increased the trust in the reliability of this modelling approach, allowing an in-depth analysis of conditions and goals of its applicability.

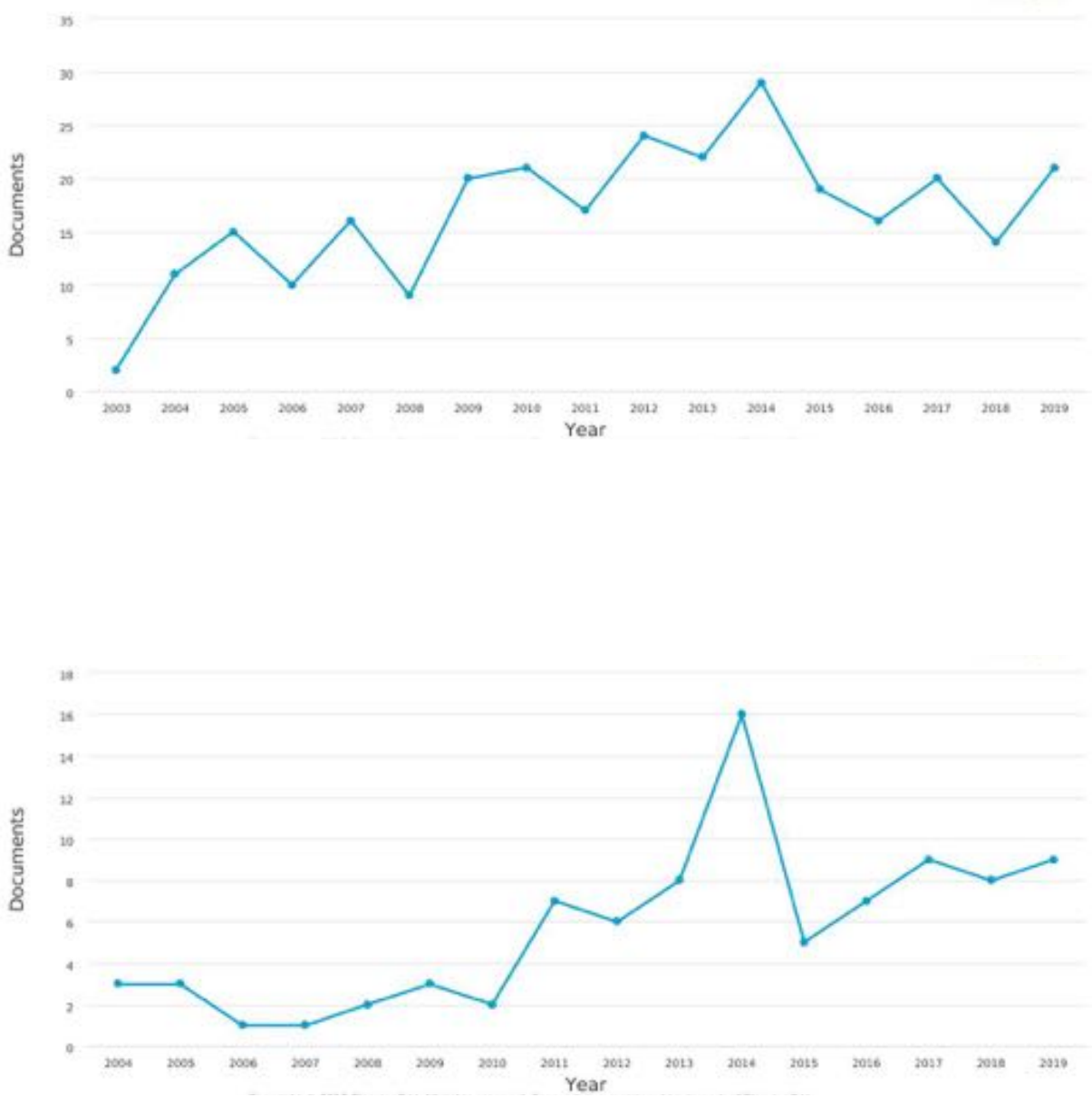

Figure 3 Citation trend of MacKenzie et al. (2002 - top) and Royle (2004 - bottom) foundational works, respectively on Occupancy and N-mixture models, in the field of Herpetology. Source of the data: Scopus, December 2019.

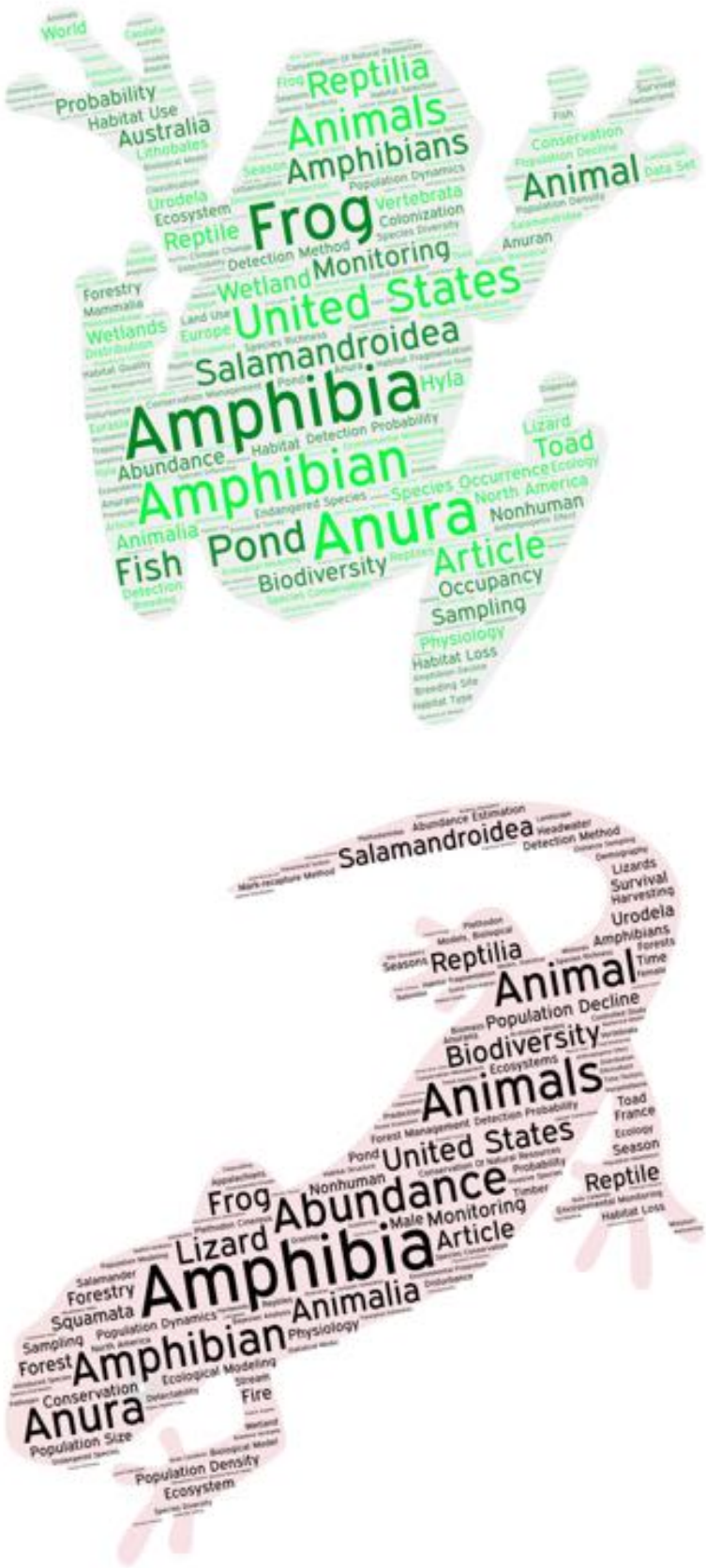

Figure 4 Word-cloud composed by the keywords of the articles citing MacKenzie et al. (2002 - top) and Royle (2004 - bottom) foundational works, respectively on Occupancy and N-mixture models, treating herpetological arguments. Font size represent the frequency of each keyword. Source of the data: Scopus, December 2019.

2. Thesis structure

The present thesis is composed by seven chapters, grouped in three distinct parts on the basis of their topic. Each one of the seven chapter is a journal article, published or produced between 2017 and 2019. Part one deals with the use of both Occupancy and N-mixture models for the conservation of salamanders in managed forests, and is composed by three chapters. Part two includes two chapters and is about the use of binomial open-population, and multinomial N-mixture models, as a tool for monitoring and estimating abundance of amphibian populations. Part three is about the reliability of count data and N-mixture models as an alternative to CMR for long term monitoring of reptile and amphibian populations, without the need of a meta-population design. This last part includes two chapters, one comparing the results of N-mixture models with those obtained from CMR, and the other one evaluating this innovative approach by means of simulations.

3. List of publications

Chapter 1 Basile, M., Romano, A., Costa, A., Posillico, M., Roger, D. S., Crisci, A., Raimondi, R., Altea, T., Garfì, V., Santopuoli, G., Marchetti, M., Salvidio, S., DeCinti, B., Matteucci, G. (2017). Seasonality and microhabitat selection in a forest-dwelling salamander. *The Science of Nature* 104, 80.

Chapter 2 Romano, A., Costa, A., Salvidio, S., Menegon, M., Garollo, E., de Fatis, K. T., Miserocchi, D., Matteucci, G., Pedrini, P. (2018). Forest management and conservation of an elusive amphibian in the Alps: Habitat selection by the Golden Alpine Salamander reveals the importance of fine woody debris. *Forest Ecology and Management* 424, 338-344.

Chapter 3 Romano, A., Costa, A., Basile, M., Raimondi, R., Posillico, M., Scinti Roger, D., Crisci, A., Piraccini, R., Raia, P., Matteucci, G., De Cinti, B. (2017). Conservation of salamanders in managed forests: methods and costs of monitoring abundance and habitat selection. *Forest Ecology and Management* 400, 12-18.

Chapter 4 Crovetto, F., Salvidio, S., Costa, A. (2019). Estimating abundance of the Stripeless tree-frog Hyla meridionalis by means of replicated call counts. *Acta Herpetologica* 14, 71-75.

Chapter 5 Costa, A., Romano, A., Salvidio, S (under review). Reliability of multinomial N-mixture models for estimating abundance of small terrestrial vertebrates. *Biodiversity and Conservation.*

Chapter 6 Costa, A., Oneto, F., Salvidio, S. (2019) Time-for-space substitution in N-mixture modeling and population monitoring. *The Journal of Wildlife Management 83, 737 – 741.*.

Chapter 7 Costa, A., Salvidio, S., Penner, J., Basile, M. (under review) Time-for-space substitution in N-mixture models for estimating population trends: a simulation-based evaluation. *Ecological Modelling.*

4. Authors' contribution

Chapter 1 MB, AR, AC and GM conceived the idea for this article. MB, AR and AC designed the sampling framework. All authors contributed to the fieldwork. MB and AC performed data analysis. MB AR and AC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 2 AR and PP conceived the idea for this article. AR, AC and SS designed the sampling framework. All authors contributed to the fieldwork. AR and SS performed CMR analyses. AC performed Occupancy analyses. AR, AC and SS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 3 AR, AC and MB conceived the idea for this article. AR and AC designed the sampling framework. All authors contributed to the fieldwork. AC performed the analysis. AR and AC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. AR and AC are co-primary authors.

Chapter 4 FC and SS conceived the idea for this article. AC designed the sampling framework. FC and SS performed the fieldwork. AC performed the analysis. SS and AC wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 5 AC and SS conceived the idea for this article. AC and SS designed the sampling framework. AC AR and SS performed the fieldwork. SS performed removal sampling analysis.

AC performed N-mixture model analysis. AC and SS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 6 AC and SS conceived the idea for this article. SS collected the data for all years. AC and FO collected the data for some years. SS performed CMR analysis. AC performed N-mixture model analysis. AC and SS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 7 AC, SS and MB conceived the idea for this article. AC performed the analysis. AC SS and MB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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PART ONE – Conservation of salamanders in managed forests

Chapter 1

Seasonality and microhabitat selection in a forest-dwelling salamander

Part of this chapter is published in: Basile, M., Romano, A., Costa, A., Posillico, M., Roger, D. S., Crisci, A., Raimondi, R., Altea, T., Garfì, V., Santopuoli, G., Marchetti, M., Salvidio, S., DeCinti, B., & Matteucci, G. (2017). Seasonality and microhabitat selection in a forest-dwelling salamander. *The Science of Nature* 104: 80.

Abstract

Many small terrestrial vertebrates exhibit limited spatial movement and are considerably exposed to changes in local environmental variables. Among such vertebrates, amphibians at present experience a dramatic decline due to their limited resilience to environmental change. Since the local survival and abundance of amphibians is intrinsically related to the availability of shelters, conservation plans need to take microhabitat requirements into account. In order to gain insight into the terrestrial ecology of the spectacled salamander *Salamandrina perspicillata* and to identify appropriate forest management strategies, we investigated the salamander's seasonal variability in habitat use of trees as shelters in relation to tree features (size, buttresses, basal holes) and environmental variables in a beech forest in Italy. We used the occupancy approach to assess tree suitability on a non- conventional spatial scale. Our approach provides fine- grained parameters of microhabitat suitability and elucidates many aspects of the salamander's terrestrial ecology. Occupancy changed with the annual life cycle and was higher in autumn than in spring, when females were found closer to the stream in the study area. Salamanders showed a seasonal pattern regarding the trees they occupied and a clear preference for trees with a larger diameter and more burrows. With respect to forest management, we suggest maintaining a suitable number of trees with a trunk diameter exceeding 30 cm. A practice of selective logging along the banks of streams could help maintain an adequate quantity of the appropriate microhabitat. Furthermore, in areas with a presence of salamanders, a good forest management plan requires leaving an adequate buffer zone around streams, which should be wider in autumn than in spring.

1. Introduction

It has been extensively shown that most small-sized terrestrial vertebrates are highly dependent on environmental conditions, such as temperature, moisture, food and shelter availability (e.g. Oatway and Morris 2007; Peterman and Semlitsch 2013; Suorsa et al. 2005). Ectothermic terrestrial species are particularly constrained to a narrow range of environmental conditions due to their physiological requirements (Wells 2007). Furthermore, since they exhibit limited spatial movement (Vasconcelos and Calhoun 2004; Rittenhouse and Semlitsch 2007; see Table 8.1 and related references in Vitt and Caldwell 2013), they are more exposed to small-scale environmental variations (deMaynadier and Hunter 1995; Popescu and Hunter 2011).

Among terrestrial vertebrates, amphibians in particular exhibit a set of physiological constraints relating to environment and microhabitat. The availability of water and an adequate level of soil moisture will influence their physiological conditions (Grover 2000; Romano and Ficetola 2010) and daily and seasonal activity (Keen 1984) and can play an essential role in determining local occurrence and abundance (Mitchell 2001; Wells 2007). Due to changes in such factors and in conjunction with other large-scale problems such as pathogens, amphibians are experiencing a global decline in their populations and a high extinction rate (Stuart et al. 2004).

Despite the small-scale habitat selection of amphibians, many conservation plans provide information and guidelines on a large or landscape scale (e.g. Baldwin et al. 2007; Trombulak and Baldwin 2010; Connette and Semlitsch 2013; Clauzel et al. 2014). This is mainly for two reasons: (i) there is scant in-depth knowledge of small-scale habitat selection and requirements of these vertebrates and (ii) most of the monitoring approaches and techniques needed to set up small-scale management plans are both time-consuming and burdensome. Among the most recent methods designed to resolve such issues, site occupancy models, which analyse presence/pseudo-absence data, permit the most direct estimate of the occupancy-related parameters (i.e. local colonisation and extinction), taking imperfect detection into account (MacKenzie et al. 2006). The above models are primarily used to map and predict large-scale animal distributions while ac- counting for biased sampling efforts (e.g. Kéry et al. 2010, Higa et al. 2014), to estimate species diversity (Dorazio and Royle 2005; see also Iknayan et al. 2014) and to improve the characterisation of meta-community structures (Mihaljevic et al. 2015).

In this paper, using an occupancy approach at a very fine spatial scale, we focused on an

amphibian with high biogeographic and conservation value, the spectacled salamander *Salamandrina perspicillata* (Savi 1821), which is a forest-dwelling, semi-terrestrial, biphasic amphibian endemic to Italy. While providing new ecological information on this species, our study also allowed us to employ and develop straightforward sampling methodology to ascertain species-habitat relationships of small terrestrial vertebrates. In recent studies in a beech forest in Central Italy, Piraccini et al. (2017) and Romano et al. (2016) showed that the number of holes between soil and stump, trunk diameter and number of buttresses are important in determining whether or not trees are used as temporary or permanent shelters in autumn by spec- tacled salamanders. In the preliminary study by Romano et al. (2016), salamander occupancy level of trees was investigated for 1 month in autumn, considering only tree features as covariates.

Our study was structured into five parts (or phases). First, we used the occupancy approach on a very fine spatial scale to investigate salamanders' microhabitat selection. Second, we investigated the annual distribution pattern of salamanders and their use of trees as shelters in three seasons (i.e. autumn and pre- and post-spawning in spring). Third, we tested whether there was a difference in spatial distribution between sexes in the different seasons. Fourth, we investigated the effect of environmental variables, considering not only those related to tree features (as in Romano et al. 2016) but also those concerning terrain features on a small scale. Fifth, on the basis of the previous findings, we suggest reliable information for forestry conservation planning.

2. Materials and methods

2.1 Study area

The research was carried out in a deciduous forest located at about 900 m a.s.l. in the Collemeluccio-Montedimezzo Man and the Biosphere UNESCO Reserve (41.76232° N, 14.21856° E, Molise, central Italy). The forest canopy is single-layered and managed as a high-growth forest. The tree species composition is dominated by *Fagus sylvatica* with sporadic *Carpinus betulus*, *Abies alba* and *Acer campestre*. *Fagus sylvatica* has a high stemflow (about 11%) compared with other tree species (Llorens and Domingo 2007). Due to its root morphology, stemflow can result in high moisture conditions in root buttress holes (codes GR11 and GR12 in the integrated catalogue of tree microhabitats: Kraus et al. 2016), as well as at the interface between buttresses and the ground. A first-order Apennine stream (i.e. with no tributaries; a headwater stream according to the Horton-Strahler ordering of streams) runs across the

study area and is used by *S. perspicillata* as a spawning site. We opportunistically selected a square study area of about 1 ha along the stream, taking account of both tree species composition and area accessibility.

2.2 Study species

The spectacled salamander *S. perspicillata* has a biphasic life cycle, with only females using streams for spawning (Angelini et al. 2007). Knowledge of the ecology of the spectacled salamander in forest ecosystems has long been fragmented and limited to the aquatic phase (Angelini et al. 2007 and references therein), despite a few recent exceptions (e.g. Romano et al. 2009; Bruni and Romano 2011; Salvidio et al. 2012; Costa et al. 2015; Piraccini et al. 2017).

2.3 Sampling methods

We selected all the trees within the study area with a diameter at breast height (DBH) ≥ 10 cm and employed Field-Map technology (http://www.fieldmap.cz—centimetre precision GPS accuracy) to obtain a distribution map of the individually labelled trees. Accordingly, each tree was considered a sampling site for our repeated surveys. Fieldwork was carried out during favourable weather conditions for salamanders—i.e. light rain or drizzle, no wind and a temperature above 8 °C (Vanni 1980; Angelini et al. 2007)—by a team of 4–7 researchers, with each sampling occasion starting 2 hours after sunrise and lasting for about 3 hours. During each survey, all trees were checked for salamander occurrence within a radius of about 1.5 m around the trunk, gently moving the litter aside and then repositioning it below the buttresses and lighting up the holes with a torch. Fieldworkers were trained in order to minimise possible discrepancies in salamander detection. The sex of salamanders was recognised following Romano et al. (2009).

2.4 Environmental variables and tree features

The environmental variables, which were unique to each site, consisted of both tree and land features. A single tree was considered to be one single site in order to derive environmental covariates from tree structure and topography. Land features were drawn from a high-resolution digital elevation model (DEM, 5 × 5 m mesh; provided by Molise University and derived by Molise Region land data). Using SAGA GIS v. 2.0.8 (Bock et al. 2011), we derived, from each mesh of the DEM, 14 variables: altitude above the stream (ABS), aspect (ASP), the water catchment area (CAR), slope of the catch- ment area (CSL), diurnal anisotropic heating (DAH), level of diffused insolation (DIN), the dominant orientation of direct insolation (DIR), duration of

insolation (DUR), slope (SLO), SAGA wetness index (SWI), topographic position index (TPI), terrain ruggedness index (TRI), total level of insolation (TSI) and topographic wetness index (TWI) (Table 1). Tree features were the same as those used by Piraccini et al. (2017) and consisted of four variables: the number of buttresses (BUT), diameter at breast height (DBH), distance from the stream (DIS) and the number of ground-level holes (HOL) (Table 1). Medians and distribution of DBH, BUT and HOL were 27 cm, 4 and 2, respectively, as detailed in Piraccini et al. (2017). Every pair of variables showed significant positive linear correlations (Piraccini et al. 2017).

2.5 Modelling and statistical analysis

The sampling design followed the same robust design as capture-mark-recapture demography studies and consisted of five primary occasions, occurring in five different months from October 2013 to October 2014, while our five secondary occasions consisted of three consecutive days, summing up to making a total of 15 sampling days (Pollock and Otto 1983; MacKenzie et al. 2003). During secondary occasions, the population is considered demographically closed, and then sites do not experience variations in occupancy. Otherwise, within primary occasions, a sampling site may be colonised or abandoned by the species. We observed no movements between trees during the secondary occasions (individual salamanders were identified by using digital pictures of the ventral pattern, see Piraccini et al. 2017 for details), leading us to assume that the population was closed during secondary occasions. Three primary sampling occasions occurred in autumn, just before overwintering (one in October 2013 and two in October 2014), when adults return to the forest and begin their court-ship behaviour (Angelini et al. 2007). One primary sampling occasion occurred in early April, during the pre-spawning phase, when females go to the stream for oviposition and males patrol the stream banks to eventually mate again (Bruni and Romano 2011). Another primary sampling occasion occurred at the end of May when, after spawning, females disperse from the stream toward the forest to overcome the dry season (Angelini et al. 2007). Therefore, the five primary sampling occasions included three different phases of the salamander's annual cycle.

We modelled four parameters of interest: the probability of a site being occupied (ψ), the probability of detection (p), the probability of a site being colonised (γ) and the probability of a site being abandoned (ε) (MacKenzie et al. 2003). We built a global model assuming ψ as a function of environmental variables, p, γ and ε as a

Code	Variable description (measure unit)	Mean	SD
• Environment			
ABS	Altitude above the stream (m)	6.15	3.90
ASP	Aspect (rad)	2.04	2.59
CAR	Water catchment area (m^2)	634.10	803.79
CSL	Slope of the catchment area (rad)	0.20	0.03
DAH	Diurnal anisotropic heating	− 0.16	0.05
DIN	Level of diffused insolation (Kwh/m^2)	0.65	0.16
DIR	Dominant orientation of direct insolation (Kwh/m^2)	0.39	0.36
DUR	Duration of insolation (h)	4.34	1.30
SLO	Slope (rad)	0.17	0.06
SWI	SAGA wetness index	8.04	1.19
TPI	Topographic position index	− 1.76	1.60
TRI	Terrain ruggedness index	0.61	0.20
TSI	Total level of insolation (Kwh/m^2)	1.04	0.50
TWI	Topographic wetness index	7.70	0.79
• Tree			
BUT	Number of buttresses	3.89	2.36
DBH	Diameter at breast height (cm)	28.34	13.74
DIS	Distance from the stream (m)	47.19	26.73
HOL	Number of ground-level holes	2.43	2.20

Table 1 Habitat variables used for modelling the probability of salamander occupancy at tree level. Environmental variables refer to a 5x5m grid covering the study area where the tree is located. Tree variables refer to the individual tree level.

function of the time variability between primary occasions. Since there are many ψ covariates, we grouped those with a Spearman rank correlation coefficient (ρs) < 0.75 and scaled them prior to analysis. As a consequence, we built several sub-global models, including just those covariates not showing high correlation, in the form of:

$\boldsymbol{\psi} \sim bernoulli(\beta0 + \beta1*covariate(1) + \beta2*covariate(2) + ... + \beta n*covariate(n))$

$\boldsymbol{p} \sim bernoulli(\beta0 + \beta1*stage(1) + \beta2*stage(2) + ... + \beta5*stage(5))$

$\boldsymbol{\gamma} \sim bernoulli(\beta0 + \beta1*stage(1) + \beta2*stage(2) + ... + \beta4*stage(4))$

$\boldsymbol{\varepsilon} \sim bernoulli(\beta0 + \beta1*stage(1) + \beta2*stage(2) + ... + \beta4*stage(4))$

The goodness of fit (GoF) of the sub-global models was tested via the Pearson chi-square test, using a parametric boot- strap procedure (5000 re-samplings) to determine whether the observed value was unusually large (MacKenzie and Bailey 2004). After selection of the candidate sub-global model, based on p values and c-hat values, model building proceeded by running every

possible combination of covariates as a function of the parameters of interest. Model selection was based on the Akaike information criterion (AIC) score (Akaike 1973), taking into account the fact that models with a ΔAIC > 2 do not have the same empirical support, and QAIC was used in the event of over-dispersion (c-hat > 1) (Burnham and Anderson 2002). Parametric analysis of variance (ANOVA) was applied, with Tukey post hoc comparison, to test whether there was a difference between annual cycle stages in the probability of a tree being occupied by salamanders. Estimated occupancy for each annual cycle stage was tested for spatial autocorrelation through the Moran test (Cliff and Ord 1981). If spatial autocorrelation was not detected, it could be inferred that trees were selected for their specific features. For the annual cycle stages that showed spatial autocorrelation, generalised least square models (GLS) were built to obtain spatially corrected estimates of occupancy (i.e. occupancy estimates were used as the dependent variable) and selected by means of the AIC score (Burnham and Anderson 2002, Beale et al. 2010). Prior to building the model, a Levene F test was applied to detect possible heteroscedasticity. Seasonal differences in the distance from the stream between males and females were tested through the Mann-Whitney test, since our data neither met the parametric assumption nor followed a complete block design. Modelling analyses were conducted using packages *unmarked* (Fiske and Chandler 2011) and *AICcmodavg* (Mazerolle 2015) in R environment, while statistical analyses were carried out with packages *nlme* (Pinheiro et al. 2015) and *spdep* (Bivand and Piras 2015).

	Total	1st autumn	Pre-spawning	Post-spawning	2nd autumn	3rd autumn
Salamanders	1012	315	208	92	269	128
Occupied trees	383	195	135	89	213	107
Observed occupancy	0.13	0.51	0.35	0.23	0.56	0.28

Table 2 Raw data of field sampling: animal and tree count

3. Results

3.1 Descriptive results

Within the study area, trees were located at a mean distance from the stream of 47.2 m (± 26.7 SD). The average value of the Spearman rank correlation coefficient among the 14 environmental variables, computed on their absolute value, was 0.35, reflecting a low correlation among covariates. Indeed, only 13 out of 91 pairs of covariates showed a ρs > 0.75 (Holm's adjusted p value < 0.01). We counted a total of 1012 individuals in the course of the whole study, with a mean of 67.5 (± 59.3 SD) per sampling occasion (Table 2). Salamanders were found at 303 trees (79%) out of 383, with a mean of 49.3 (± 32.7 SD)

occupied trees per sampling occasion (Table 2).

3.2 Results from occupancy models

The sub-global model with the probability of occupancy (ψ) constrained by ASP, BUT, DAH, DBH, CSL, DIS, HOL, TRI and TWI was selected for model building (global p value = 0.08; c-hat = 1.5). The parameter estimates of the probabilities of detection (p), colonisation (γ) and abandonment (ε) were modelled as a function of seasonality, i.e. the parameters were allowed to change between the three annual cycle stages. Model building resulted in 2048 models and the QAIC score was used for selection due to c-hat > 1. The six models ranked ΔQAIC < 2 are reported in Table 3. However, a more conser- vative approach was adopted, with three models ranking ΔQAIC < 1 (Table 3). Model averaged estimates were:

$\psi = 2.88 - 0.22*CSL + 1.59*DBH - 0.32*DIS + 1.42*HOL$

$p = -1.28(autumn) - 0.04(pre\text{-}spawning) - 0.91(post\text{-}spawning)$

$\gamma = -8.85(autumn) + 8.84(pre\text{-}spawning) + 3.49(post\text{-}spawning)$

$\varepsilon = -0.68(autumn) - 7.96(pre\text{-}spawning) - 9.06(post\text{-}spawning)$.

The best model suggested that the probability of occupancy is a function of DBH and HOL, while, in the other empirically supported models, the probability was also constrained by DIS (ΔQAIC = 0.26) and CSL (ΔQAIC = 0.85; Table 3). The proportion of the area occupied (PAO, corresponding to the proportion of trees occupied) estimated by the best model was greater in the first autumn (PAO = 0.82, CI95% = 0.56–1) and lower in the third autumn, at the start of the wintering phase (PAO = 0.49, CI95% = 0.23–1; Table 4). The probability of detection increased to the maximum value at the beginning of spring, in the pre-spawning phase (p = 0.50, SE = 0.04), while it was

Table 3 Estimates of occupancy model parameters and QAIC scores

Model parameters	β estimates (SE)	QAIC	ΔQAIC	QLL
i	3.00 (0.63)**	2836.32	0	− 1405.158
DBH	1.69 (0.55)**			
HOL	1.44 (0.66)*			
i	2.80 (0.58)**	2836.58	0.26	− 1404.287
DBH	1.49 (0.53)**			
HOL	1.42 (0.66)*			
DIS	− 0.32 (0.23)			
i	2.80 (0.56)**	2837.17	0.85	− 1404.582
DBH	1.56 (0.53)**			
HOL	1.39 (0.63)*			
CSL	− 0.22 (0.28)			
i	3.01 (0.66)**	2837.42	1.11	− 1404.71
DBH	1.71 (0.57)**			
HOL	1.48 (0.67)*			
TRI	0.11 (0.21)			
i	2.83 (0.60)**	2837.54	1.22	− 1403.77
DBH	1.63 (0.56)**			
HOL	1.30 (0.70)			
ASP	− 0.25 (0.26)			
DIS	− 0.38 (0.23)			
i	2.80 (0.59)**	2838.15	1.84	− 1404.08
DBH	1.48 (0.53)**			
HOL	1.44 (0.67)*			
CSL	0.07 (0.33)			
DIS	− 0.37 (0.30)			

Estimates are based on standardised covariates

DBH tree diameter at breast height, *HOL* number of ground-level holes, *DIS* distance from the stream, *CSL* slope of the water catchment area

*p value < 0.05

**p value < 0.01

Parameter	1st autumn	Pre-spawning	Post-spawning	2nd autumn	2rd autumn (pre-wintering)
ψ	0.82 (0.03)	0.55 (0.07)	0.78 (0.08)	0.78 (0.04)	0.49 (0.05)
p	0.22 (0.01)	0.50 (0.04)	0.29 (0.03)	0.22 (0.01)	0.22 (0.01)
γ		1 (0.003)	0.97 (0.77)	0.0002 (0.003)	0.0002 (0.003)
ε	0.34 (0.06)	0	0	0.34 (0.06)	

Smoothed ψ is given by non-parametric bootstrap (1000 re-sampling) of the SE. p, γ and ε have been considered similar for the first, second and third autumns, resulting in similar estimates

ψ occupancy, p detectability, γ colonisation, ε extinction

Table 4 Estimates of parameters and SE in the different seasons

lower during the autumn (p = 0.22, SE = 0.01; Table 4). However, only the spring βestimate of detectability was not significant (p = 0.92). Similarly, β estimates of colonisation and abandonment were non-significant, with the exception of the estimate of aban- donment in autumn (p = 0.61, autumn; p = 0.61, pre- spawning; p = 0.90, post-spawning; p = 0.98, pre-spawning; p = 0.97, post-spawning). Analyses of variance showed major differences in single- tree occupancy in different annual cycle stages (d.f. = 4; F = 134; p value < 0.01). Pairwise post hoc tests were significant for every comparison (p value < 0.05), with the exception of the post-spawning stage vs. the second autumn (p value = 0.98). Consequently, the probability of occupancy of each tree changed between annual cycle stages, as a function of covariates, with high values occurring throughout the year except when the salamanders were closer to the stream in the early spring (Fig. 1). Single-tree occupancy values were auto-correlated in space in the pre-spawning stage (Moran I = 0.20; p value < 0.01) and in the third autumn occasion (Moran I = 0.04; p value < 0.014), while no spatial autocorrelation was detected in the other life cycle stages (p = 0.37, 1st autumn; p = 0.06, post-spawning; p = 0.09, 2nd autumn). Indeed, the stream-oriented pattern of animal movements became evident during the pre-spawning stage due to the change in overall tree occupancy according to distance from the stream (Fig. 2). Therefore, GLS analysis was only applied to the pre-spawning stage. Sixteen GLS models were built, starting from the null model and combining the four variables selected from occupancy modelling (i.e. CSL, DBH, DIS, HOL). The model that scored the lowest AIC value was that with all four covariates (AIC = − 691.89; AICweight = 0.68). However, DBH and HOL showed higher estimates (βDBH = 0.15; βHOL = 0.13; p values <

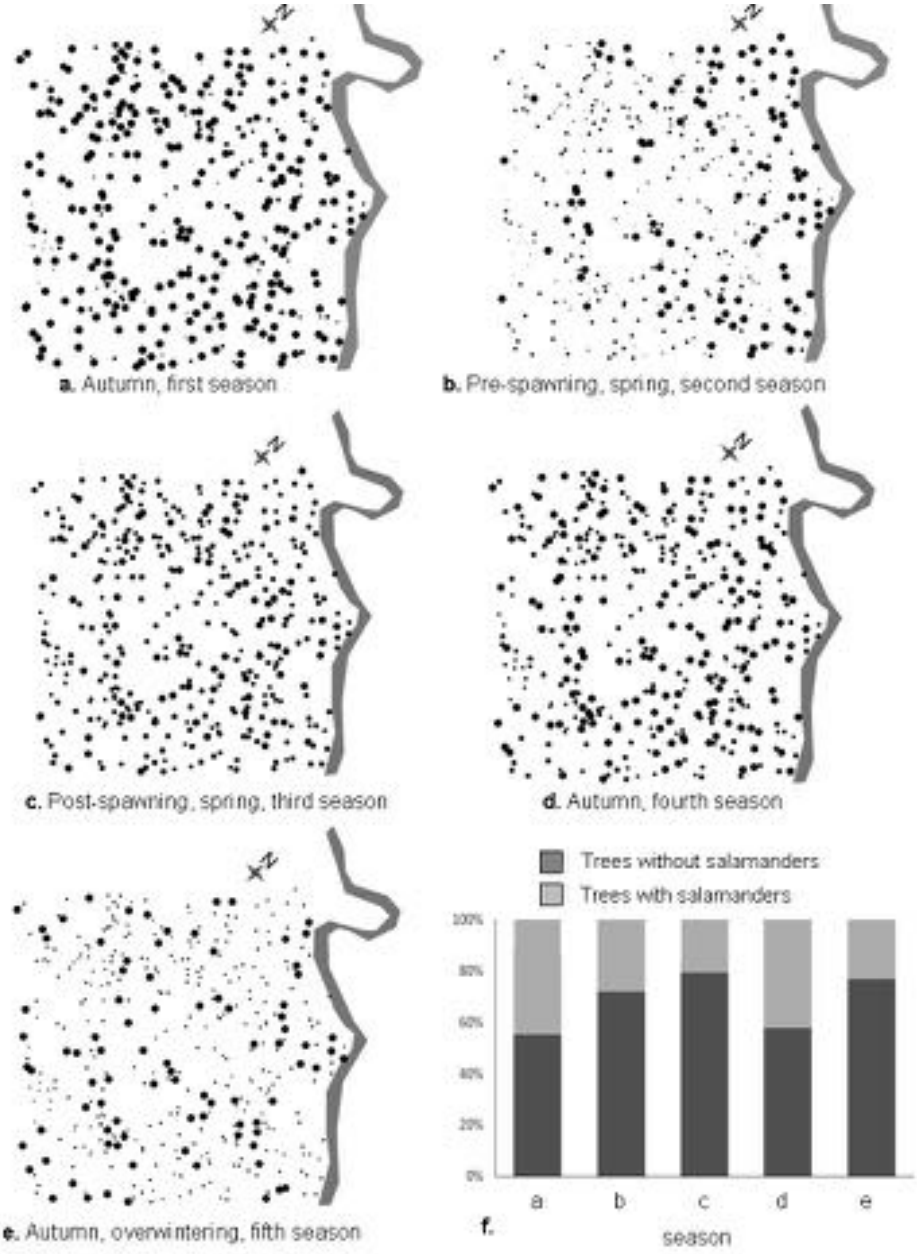

Figure 1 (a to e) Probability of occupancy at the single-tree level in different seasons; (f) seasonal percentage of trees hosting or not hosting salamanders. Circles represent the 383 trees with DBH>10cm. Circle width is proportional to the probability of occupancy.

0.01) than CSL and DIS (βCSL = 0.007; βDIS = −0.008; p values < 0.05), indicating a greater influence of diameter and ground-level holes in determining occurrence at each tree, as in the occupancy model (Fig. 3).

3.3 Spatial distribution of salamanders

The pattern of spatial distribution between sexes varied with the annual cycle. Males and females were only found at a different distance from the stream in the pre-spawning stage, females being significantly closer to the stream than males (mean ± SD: 21.0 ± 16.7 and 31.7 ± 25.6 m, respectively; U = 1337; p value = 0.04), and the observed sex

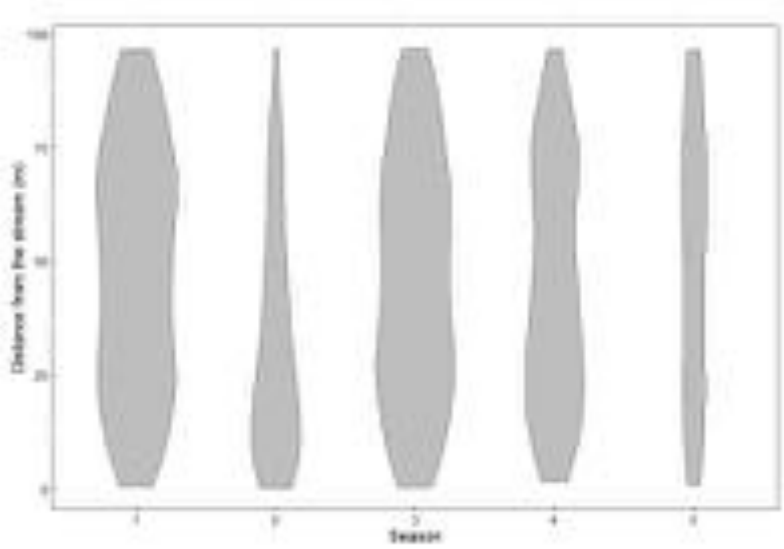

Figure 2 Relative abundance of occupied trees as a function of distance from the stream. Violin width is proportional to the number of trees for which $\psi \geq 0.7$. 1 = first autumn; 2 = spring/pre-spawning; 3 = spring/post-spawning; 4 = second autumn; 5 = second autumn before wintering

ratio favoured males in every life cycle stage except for late spring (Fig. 4).

4. Discussion

Shelter availability is fundamental among amphibians, especially for those that mainly rely on their skin for gas exchange, such as lungless salamanders (Plethodontidae; Keen 1982) and *Salamandrina*, which only possess rudimental lungs (Anselmi 1921). Many biphasic salamanders are affected in their reproduction by seasonal environmental conditions, but spend most of the year in terrestrial environments where they are influenced by microclimate conditions (Welsh 1990; deMaynadier and Hunter 1995; Grover 2000). Environmental gradients (e.g. moisture) and/or the availability of suitable microhabitats can exacerbate the seasonal oscillations in abundance, occurrence or patterns of activity in salamanders (Faccio 2003; Bailey et al. 2004). The spectacled salamander shows a seasonal pattern of activity influenced by its latitudinal location and microclimate conditions (Utzeri et al. 2004; Angelini et al. 2007). Our results showed that the slope of the water catchment area and the distance from the stream were not significant. They were chosen in model selection, indicating a certain degree of influence on salamander occurrence. These two variables are intuitively related to soil moisture and the availability of moist burrows. This pattern may be attributed to the greater importance of the other two variables selected (i.e. trunk diameter and the number of holes), which can play an essential role in microhabitat selection. Detectability was relatively low in every season, except in spring due to the higher level of activity caused by breeding migration. After spring, the individuals returned to the forest to disappear later as winter approached. There is a clear discrepancy between the estimated proportion of trees occupied and observed

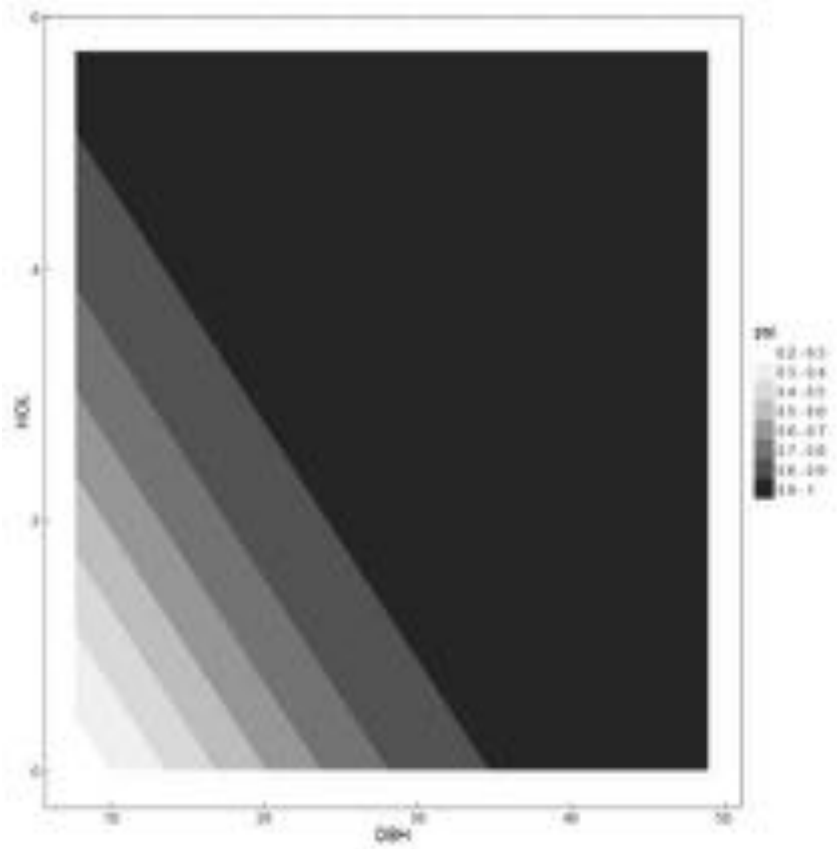

Figure 3 Effect of diameter (DBH) and number of holes (HOL) in affecting the probability of occupancy of trees.

occupancy. A rapid return to the forest after the spawning season (late May) and greater use of shelters because of the need for protection from the advancing dry season may represent the effects of the accentuated discrepancy in the post-spawning season. Furthermore, the estimates for this season are similar to those obtained in autumn, indicating a renewed widespread population. Finally, albeit still widely distributed, the population begins to disappear in late autumn as spectacled salamanders spend the winter up to 20 cm below the soil surface (Angelini et al. 2007). Piraccini et al. (2017) showed the first evidence of an association between *Salamandrina* occurrence and tree trunk diameter, buttresses and holes in autumn. We developed the present study from that starting point, using count data instead of captures, collected in the same reference site, to assess the occupancy dynamic over the year. As in Piraccini et al. (2017), DBH is clearly the most important variable for a tree to be considered as suitable habitat, and it can determine, in conjunction with the number of holes, not only how a tree is exploited (i.e. permanent, temporary or no shelter) but also how the population occurrence changes in the course of the year. This strong relationship had already emerged in Romano et al. (2016), where the use of trees as shelters was investigated for autumn alone. We extended that approach in order to gain year-round information on the spectacled salamander, tree selection during three biologically

different seasons, the influence of small-scale land features and sexual differences in the use of trees. The overall absence of spatial autocorrelation among occupancy estimates supports our hypothesis that spectacled salamanders select the most suitable trees. Both our work and that of Piraccini et al. (2017) revealed that spectacled salamanders prefer larger trees and that there was no spatial autocorrelation in the use of trees in autumn (this work, Piraccini et al. 2017). However, we observed that salamanders can also be encountered on small trees, next to larger ones, in spring. This, albeit low, spatial autocorrelation in spring can be explained by migration, which leads to a higher density of salamanders closer to the stream, as well as by more flexible behavior adopted by spectacled salamanders. Indeed, salamanders of both sexes may migrate from one tree to another using small trees as stepping stones, or salamanders of both sexes may concentrate near the stream for mating (Bruni and Romano 2011). In a situation of high density of salamanders, there could be competition for larger shelters, although experimental studies did not not detect any territorial competition between females during the spawning season (Romano and Ruggiero 2008), while there is a lack of information about territoriality in males which shows intra-sexual aggressive behavior (Bruni and Romano 2011). However, if there is intraspecific competition, smaller individuals or those with a lower body condition (Costa et al. 2015) should be observed sheltering near smaller trees.

4.1 Implications for conservation

Terrestrial salamanders are mainly associated with forest habitats (Wells 2007). The alteration of such habitats, e.g. by intensive thinning, may have marked negative effects, such as a reduction in both the abundance and dispersal capability of these salamanders (Grialou et al. 2000; Patrick et al. 2006). However, to date, research efforts have focused on the stand level, ascribing the trends of abundance or occupancy to changing stand characteristics (Semlitsch et al. 2009; Mcintyre et al. 2012). By contrast, our research focused on a smaller scale by investigating the features of individual trees within the stand in order to provide fine-grained proxies of tree suitability as salamander shelters by means of occupancy level. Occupancy models are particularly useful with species that are cryptic and/or difficult to sample or with species that are cryptic and/or difficult to sample or with species whose presence may be detected without captures (Ball et al. 2005; Durso et al. 2011). However, even though extensive research has been produced to be applied to natural resource management, there remains a gap between

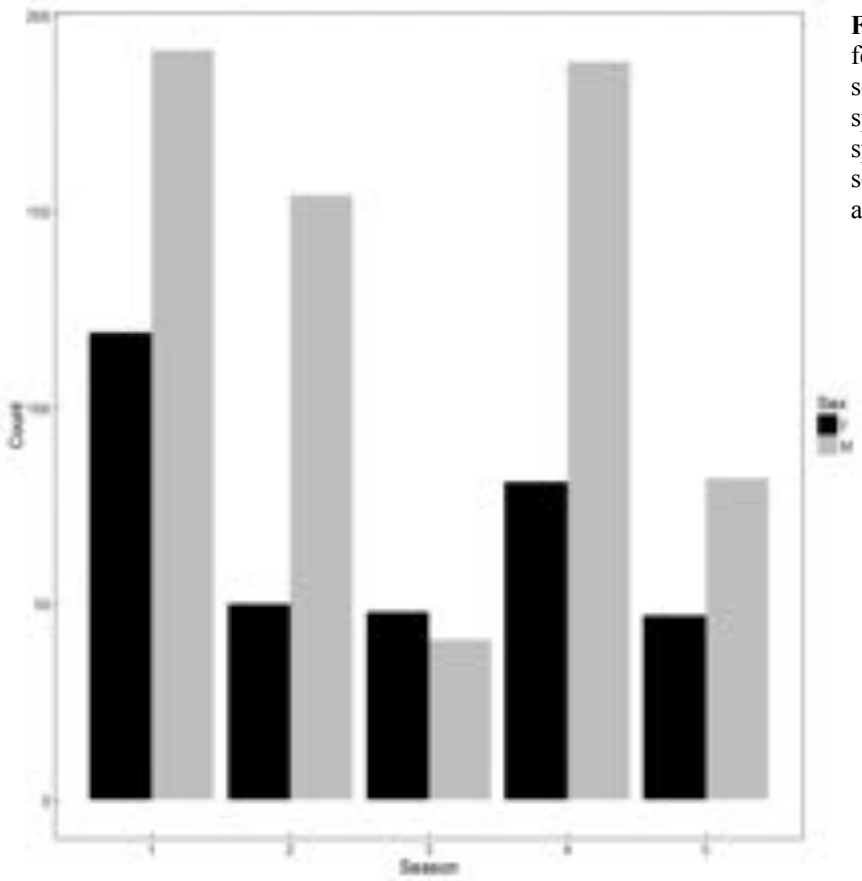

Figure 4 Number of males and females detected in every season. 1 = first autumn; 2 = spring/pre-spawning; 3 = spring/post-spawning; 4 = second autumn; 5 = second autumn before wintering

theoretical and practical conservation (Hulme 2011; Griffiths et al. 2015). On the strength of the findings in our case study, using our fine-scale and multi-seasonal occupancy framework, we are able to propose easy-to-use guidelines for conservation practitioners which are in agreement with the results published by Piraccini et al. (2017), especially concerning the importance of retaining an appropriate number of trees with a DBH > 30 cm or keeping a higher portion of such trees than what is usually the practice. We suggest that retaining the stump (and its cavities) after logging, and not preserving the whole tree, is an inadequate conservation measure because local conditions suitable for salamanders (e.g. shade, temper- ature, moisture) would change markedly due to the absence of stemflow and higher direct solar exposure. In fact, a large number of individuals at sites can result in high occupancy even if the probability of selection is small (Lele et al. 2013). In our case, there were trees with a significantly low occupancy despite the large number of individuals observed in the whole forest, which further demonstrates salamander selection of specific trees on the basis of structural features. Indeed, we showed that this is a useful approach in assessing single-tree selection by animals. Appropriate tree and land features that have to be analysed may vary among animal taxa, but the general

approach adopted here, using occupancy on a small scale and trees as sampling units, may be a very useful tool for other forest-dwelling vertebrates and invertebrates which are associated with single trees. Another management implication derived from understanding animals' seasonal activity is the temporal and spatial planning of logging activities. While logging should be avoided in autumn or conducted at a considerable distance from streams, logging in spring can be carefully planned by taking into consideration both the presence of suitable (large) trees in the area and the proximity of a reproductive site. A buffer of 60 m (twice the male dispersion distance) around the reproductive site is advised, as well as selective harvesting of trees based on DBH. Animal conservation and habitat management are intertwined with human-influenced landscapes and it is reductive to treat these subjects as separate issues. Our work can be seen as an effort to bridge the gap between ecologists, forest managers and conservation practitioners by integrating a novel ecological modelling approach to reveal animal ecological traits in order to guide conservation.

Aknowledgments

This study was supported by the Life project, ManFor CBD LIFE09 ENV/IT/000078 (Managing Forests for multiple purposes: Carbon, Biodiversity and socio-economic well-being). All the experimental protocols were approved by the Italian Ministry of the Environment with the authorisation number PNM-II-2012-0015691. We are grateful to Rodolfo Bucci, Filippo La Civita, Rosario Balestrieri, Giovanni Capobianco, Salvatore Ferraro, Valeria Balestrieri, Riccardo Piraccini and Riccardo Novaga for their contributions to field sampling. Domenico De Vincenzi (Ufficio Territoriale per la Biodiversità di Isernia—Corpo Forestale dello Stato), and the staff at the Posto Fisso di Montedimezzo and Azienda Sperimentale Demaniale Torre di Feudozzo, contributed greatly by providing logistical facilities and accommodation close to the sampling site, as well as help and materials to carry out the sampling. Thanks are due to Martin Brimble and Mark Jonathan Walters for language revision. We would like to thank the two anonymous reviewers for their suggestions and comments.

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Chapter 2

Forest management and conservation of an elusive amphibian in the Alps: habitat selection by the Golden Alpine Salamander reveals the importance of Fine Woody Debris

Part of this chapter is published in: Romano, A., Costa, A., Salvidio, S., Menegon, M., Garollo, E., de Fatis, K. T., Miserocchi, D., Matteucci, G., & Pedrini, P. (2018). Forest management and conservation of an elusive amphibian in the Alps: Habitat selection by the Golden Alpine Salamander reveals the importance of fine woody debris. *Forest Ecology and Management* 424: 338-344.

Abstract

Amphibians are declining worldwide and one of the major causes of such decline is habitat loss. Forestry practices have a primary role in causing habitat loss and fragmentation, detrimental to amphibians. We studied the ecological requirements of a fully terrestrial and threatened amphibian, the Golden Alpine Salamander *Salamandra atra aurorae,* which is endemic to a small portion of the Italian Alps. This rare and elusive salamander lives exclusively in forest environments and forestry practices are considered among its major threats. We employed both a capture-mark-recapture (CMR) and an occupancy approach in fifty 400 m^2 plots, within a managed mixed forest dominated by Norway spruce and to a lesser extent beech, and silver fir. Modelling salamander occupancy as a function of site-specific habitat features allowed us to understand the ecological requirements of this salamander and provide precise guidelines for forest management. The application of hierarchical models (occupancy) for evaluating forest management plans is highly effective, requires less effort and is ~~a~~ less impacting methodology than CMR performed by searching for salamanders under shelters also in non-optimal weather conditions. Distance from open pasture edges significantly affects the distribution of salamanders while, at a smaller scale, brushwood piles, classified as fine woody debris (FWD, diameter from 1 to 10 cm), play a key role in providing suitable habitat for this endangered amphibian. The importance of FWD in the conservation of small vertebrates is generally poorly studied and probably underestimated. However, our results show that FWD should be considered as an additional element that has to be managed to enhance habitat suitability for this and, intuitively, for other small forest vertebrates.

1. Introduction

Anthropogenic causes, especially habitat change and degradation, are the main factors resulting in global biodiversity loss (Newbold et al. 2015). Populations trends and species extinction in amphibians suggest their systematic and dramatic decline worldwide, making them the most endangered class of vertebrates (Stuart et al. 2004; Leung et al. 2017). Amphibian populations are declining, even in temperate regions, such as North America and Europe where stringent environmental regulations are usually implemented (Leung et al. 2017). Among the human activities resulting in habitat fragmentation, modification and loss, unsustainable forestry may alter the habitats both at a global (Carlson & Groot 1997) and fine scale level (Riffel et al. 2011). Although most
amphibian species have biphasic lifestyles (i.e., aquatic larvae and terrestrial adults), several of them are completely terrestrial and associated to forest environments (Petranka 1998). Therefore, protection and sustainable management of woodlands is the only way to preserve amphibian populations of these fully terrestrial species.

In particular, terrestrial salamanders are highly constrained to a narrow range of environmental conditions due to their physiological requirements (Feder 1983; Peterman & Semlitsch 2014). Moreover, they exhibit limited spatial movements (see Table 8.1 and related references in Vitt & Caldwell 2013) and they are highly sensitive to small-scale variation in soil moisture and shelter availability (deMaynadier & Hunter 1995; Popescu & Hunter 2011; Peterman & Semlitsch 2013). However, many management plans with multiple aims (e.g. forest productivity and biodiversity conservation) provide information and guidelines only at the landscape level (Trombulak & Baldwin 2010; Connette & Semlitsch 2013; Clauzel et al. 2014). At smaller scales, the best studied aspect linking herpetofauna conservation and forest management is the retention of deadwood in harvested areas (see Otto et al. 2013 and references therein). When appropriate forest management strategies are set up to target terrestrial amphibians, knowledge of their ecological features is a prerequisite. A common issue with such species is that they are often difficult to detect. Detection plays a key role in determining the presence or (supposed) absence of a species at a site. However, site occupancy models accounting for imperfect detection (MacKenzie et al. 2017) allow estimates of the occupancy parameter (i.e. the probability that a species is present at a site).

Recently, site occupancy models applied at small spatial scales have provided useful information for conservation and

management of forest salamanders (Basile et al. 2017; Romano et al. 2017). We applied this approach to a fully terrestrial and endangered salamander strictly associated to alpine forest habitats, the Golden Alpine salamander Salamandra atra aurorae Trevisan, 1982. The study area has been subject in recent decades to forestry exploitation. Our aim was to gain insight into the ecological requirements of this endangered salamander, in order to provide the first practical guidelines for its conservation in managed forests.

2. Materials and methods

2.1 Study taxon

Salamandra atra aurorae, the Golden Alpine Salamander, is one of the four recognised subspecies of the Alpine salamander *S. atra.* Like other Alpine salamanders, it is a fully terrestrial taxon and females, after a 2-3 year gestation period, give birth to just one or two fully developed young (Bonato & Fracasso 2000). This salamander is endemic to northern Italy, occurring in a small portion of the south-eastern Prealps (Riberon et al. 2001; Bonato & Steinfartz 2005). It is restricted to montane forests covering part of the Sette Comuni plateau in the province of Vicenza (Veneto region) and Trento (Trentino Alto Adige region). This amphibian has a declining population trend (Grossenbacher 1995), and its restricted geographic distribution (area of occupancy is less than 20 km^2) and potential threats are the main reasons for its current endangered status and difficult assessment (e.g. Romanazzi et al. 2012). In particular, forestry practices, that in recent years has sometimes led to massive use of heavy equipments, responsible for compacting the soil, eliminating cavities and potential shelters, are considered among the threats that could drive this salamander to extinction in a very short time period, as assessed by the Italian IUCN Red List. This salamander is also included as "priority taxon" in Annex II of the European Union Directive 92/43/EEC, known as the "Habitats Directive", which is the most important biodiversity legislation implemented in Europe. Despite its high conservation value, information on the ecology of *S. atra aurorae* is scanty. Preliminary data on its activity suggested that structural features of the soil surface and forest composition are significant variables influencing its presence, with silver fir (*Abies alba*) and beech (*Fagus sylvatica*) forests offering a more suitable habitat than plantations of Norway spruce (*Picea abies*) (Bonato & Fracasso 2015).

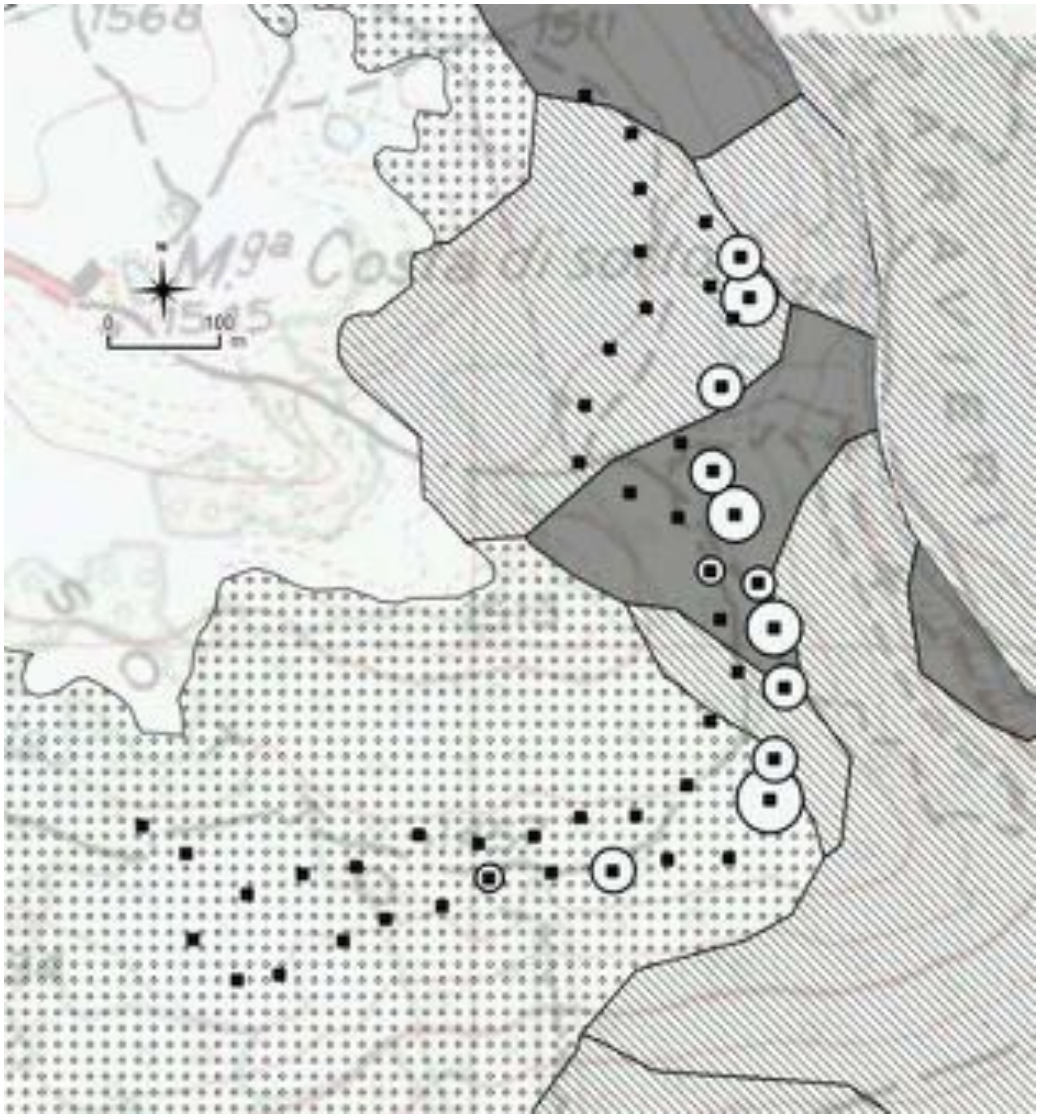

Figure 1 Spatial distribution of sampling sites (black squares) of Golden Alpine Salamander on the Vezzena plateau (Northern Italy). Abundance of salamanders (from 1 to 6 records) in each plot is proportional to the size of white circles. Dotted area: Norway spruce; grey area: Silver fir; shaded area: Beech and Silver fir; blank area: open pasture

2.2 Study area, study framework and sampling design

The study area is a small portion of the species range, located on the Vezzena plateau (Trentino Alto Adige region; 45°57'10"N, 11°22'25" E) at about 1450 m a.s.l.. The Vezzena plateau falls within the general climatic context of an Alpine mountain region. According to Thornthwaite & Mather's classification (1955, 1957) the climate of this area can be assigned to a mesothermic-to-microthermic, perhumid type, with no significant dry periods. In the study site, alpine pastures, silver fir, beech and plantations of spruce dominate the forest landscape.

We identified, delimited, GPS-positioned and individually labelled 50 square plots, following a systematic design (i.e. sampling plots are selected according to a random starting point and a fixed periodic interval).

Each plot measured 400 m^2 (20x20 m). Plots were considerably larger than the home range of this salamander (calculated by Bonato & Fracasso 2003 using the maximum distance between two capture points - mean 8 m, range 0-32 m - as a rough estimate of the width of the annual home ranges). Plots were located along two sub-parallel transects above and below a forest track (Fig.1). Twenty-five of them were in a spruce-dominated forest, 10 were placed where silver fir was dominant and 15 were in a mixed forest with silver fir and beech (Fig.1). The two transects were distant from 30 to 100 m apart and the distance between plots along a transect was about 50 m [mean distance between plots was 45 (7.9) m, range = 24-62 m]. Thus, we had a total sampled area of 2.0 ha, included within a forest stand of 9.5 ha.

We performed both an occupancy approach and a capture-mark-recapture (CMR) study in all the 50 plots. Salamanders captured inside the plots were individually identified using digital pictures of the dorsal pattern, which is unique and persistent for each individual in this species (Bonato & Fracasso 2003), at least throughout the brief study period (Steinfartz 1998). As we observed no salamanders moving from one plot to another during the sampling period, we assumed that the individuals of this population had a home range which was narrower than our plot size (according to Bonato & Fracasso 2003) and were active in a limited area. The same plots were used as sites for our spatially and temporally replicated occupancy surveys (i.e. observers recorded the detection/non-detection of salamanders).

During summer 2017 (end of June - beginning of July) all sites were visited seven times by 4-8 observers. During each survey two observers per plot sampled a site for four minutes before moving to the next sampling site. Each survey lasted about 2-3 hours, considering both searching for salamanders under shelters and detecting salamanders by walking on the forest floor within each plot. For occupancy analyses we used only data obtained from surveys performed during optimal climatic conditions for salamanders (*i.e.* wet or slight rain and under limited wind; three surveys in all) in order to maximise the probability of detecting active salamanders and to minimise stochastic heterogeneity in detection histories (Lefosse et al. 2016). By contrast, for CMR we used data obtained from all seven surveys. Salamander sex was assessed by direct observation of the cloacal region, which is swollen in the males, or of the evidence of pregnancy (Klewen 1988).

2.3 Data analysis

For each plot we measured six site-dependent and structural variables. Most

variables were recorded in the field, while one was derived from GIS. In the field we recorded the total number of trees for each plot (TREES), specifying the number of trees for the three more frequent species: *Abies alba* (ABIES), *Picea abies* (PICEA) and *Fagus sylvatica* (FAGUS), and derived the relative abundance of the most common species (i.e. *A. alba;* ABIES). In each plot we also measured the forest floor surface (m^2) occupied by rocks (ROCK) and brushwood piles (BRUSH), with a precision of 5 m^2. We also counted the number of dead wood chips longer than 30 cm and wider than 10 cm (WOOD). These variables give information on both forest structure (Weller 1987) and availability of shelters for forest salamanders (Piraccini et al. 2016; Basile et al. 2017). Finally, we measured the minimum distance from the centre of each plot to the forest edge (EDGE) using QGIS Software. In a preliminary occupancy analysis, we also evaluated several covariates derived from a digital elevation model (DEM; 5 m resolution) representing the microclimatic conditions capable of explaining the occurrence of salamanders (Peterman & Semlitsch 2013). In particular, using SAGA GIS v. 2.0.8 (Bock et al. 2011), we calculated three covariates for every cell enclosed in each plot: the Topographic Wetness Index, the Aspect of the slope and the Terrain Ruggedness Index. However, since the effect of these covariates was not significant (i.e. this whole data-set did not pass a goodness of fit test), we excluded them from subsequent analyses.

For the analysis of our repeated detection/non-detection data we employed site occupancy models: accounting for imperfect detection, such models allow the proportion of area occupied by the species to be estimated (MacKenzie et al. 2002). Site occupancy models provide estimates of two parameters: the state variable, i.e. the probability of the species being present at the site (occupancy; Ψ), and the detection probability (p), i.e. the probability of detecting the species, given it is present at the site (MacKenzie et al. 2017). Approaching data analysis, as a first step, we scaled and standardized our covariates (i.e. mean = 0, SD = 1; all covariates are continuous) and then we conducted a correlation analysis between them in order to identify possible collinearity (MacNally 2002). Then, we proceeded to build a global model, i.e. the model including all the covariates and in which other candidate models are nested, and we assessed the goodness of fit of this model by means of a Pearson Chi-Square test using a parametric bootstrap procedure (5000 re-sampling; MacKenzie & Bailey 2004). From the global model we built all possible candidate models, derived from different combinations of covariates and detection probability structures. Occupancy was modelled as a

function of site-specific covariates, while the detection probability was considered to be constant over time or survey-dependent. Model selection was conducted according to the quasi-likelihood counterpart of Akaike's Information Criterion, adjusted for over-dispersion (c-hat) and small sample size (i.e., QAICc; Akaike 1973; Burnham & Anderson 2002), considering that models with a ΔQAICc > 2 show substantial differences (Burnham & Anderson 2002). As sample size we used the number of sites which is a wide employed option (Mazerolle et al., 2011). All statistical analyses were performed in R environment with packages Unmarked (Fiske & Chandler 2011), MuMin (Barton 2009) and AICcmodavg (Mazerolle 2011).

For estimating abundance, we first performed the closure test using the software CAPTURE (Otis et al. 1978) and, since the population was found to be closed, we used the same software to estimate the abundance of salamanders during our survey period. We considered the following models for closed populations (White et al. 1982; Pollock et al. 1990): the equal catchability model (M0), the heterogeneity model (Mh), the behaviour model (Mb), the time variation model (Mt) and other models based on different combinations of the three main sources of variation in capture probabilities (Mbh, Mth, Mtb , Mtbh). Goodness of fit of each model, selection of the best model and estimation of population size were performed as implemented in the software CAPTURE (Otis et al. 1978; White et al. 1982).

3. Results

3.1. Population density

During the seven surveys, we encountered 40 Golden Alpine salamanders in all (from 0 to 3 salamanders/plot) which were attributable to 33 individuals recognized by the unique dorsal pigmentation patterns (18 males, 15 females). Salamanders were detected in 13 sites (26% of the total plots) which were not uniformly distributed (Fig.1). The statistical procedure performed by the program CAPTURE selected the time variation model (Mt) as the best fitting model and the suggested estimator was Darroch, which sets the probability of not being caught during the study equal to the product of the probability of not being caught during each respective sampling period (Darroch 1958). Capture probability varied between 0.03 and 0.15 (Tab.2). The estimated

Candidate models in ΔQAICc < 4 range

	Model	Parameters	QAICc	ΔQAICc	*Wi*
m1	p(.) ψ(BRUSH+EDGE)	4	84.2	0.00	0.30
m2	p(.) ψ(BRUSH+EDGE+TREE)	5	86.3	2.14	0.10
m3	p(.) ψ(BRUSH+EDGE+WOOD)	5	86.4	2.23	0.09
m4	p(.) ψ(BRUSH+EDGE+ROCK)	5	86.5	2.32	0.09
m5	p(.) ψ(BRUSH+EDGE+ABIES)	5	86.7	2.49	0.03

Relative variable importance (W*t*)*

Variable	W*t*
BRUSH	0.61
EDGE	0.61
WOOD	0.10
TREE	0.10
ROCK	0.09
ABIES	0.03

Ψ Estimates (SE) for models in ΔQAICc < 4 range

Mod.	Intercept	*β* BRUSH	*β* EDGE	*β* WOOD	*β* TREE	*β* ROCK	*β* ABIES
m1	-1.81(0.70)	1.55(0.68)	2.17(0.93)	-	-	-	-
m2	-1.73(0.68)	1.51(0.73)	1.97(1.00)	-	0.34(0.48)	-	-
m3	-1.88(0.72)	1.46(0.69)	2.2(1.01)	0.31(0.48)	-	-	-
m4	-1.98(0.83)	1.56(0.68)	2.59(1.33)	-	-	-0.34(0.63)	-
m5	-1.86(0.75)	1.58(0.69)	2.25(1.00)	-	-	-	0.18(0.54)

Table 1 List of candidate models laying within ΔQAICc < 4; Relative variable importance, calculated as the sum of the QAICc weights of the models including the variable; Estimates of the state variable for the listed models.

number of individuals was 79 (23.2) (95% C.I. = 52-150). Considering the whole area surveyed (2 ha) salamanders showed a density of 39 individuals/ha (95% C.I. = 28-50).

3.2. Occupancy level and ecological outcomes

Although under the occupancy approach we only considered salamanders found in three surveys, in optimal climatic conditions, the plots with ascertained presence of salamanders were the same as those emerging during the seven surveys used for the CMR study, i.e. 13 plots. During the three occupancy surveys we encountered a total number of 30 salamanders (8, 10 and 12 respectively). Spearman's rank correlation coefficient for site-specific covariates indicated a lack of significant correlation ($\rho s < 0.7$; $P > 0.05$) allowing us to include all covariates in the global model (Dormann et al., 2012). The MacKenzie & Bailey (2004) goodness of fit test for our global model resulted in a good fit ($P = 0.31$) and low over-dispersion (c-hat = 1.1). Among the 126 candidate models built from combinations of covariates and detection probability structure the best models laying within ΔQAICc < 4 (Table 1) accounted for constant detection probability. From the best model (m1 in Table 1) we obtained an overall occupancy estimate of 0.28 (95% C.I. = 0.20 – 0.37), which suggests that 14 out of 50 sites are occupied by the species (95% C.I. = 10 – 19), and a detection probability of 0.63 (95% C.I. = 0.46 – 0.77).

This model (Beta estimates for this model and for the others are reported in Table 1) also suggests that the probability of occupancy is positively influenced by the amount of brushwood piles, that in each plot (400 m2) covered a surface ranging between 0 and 110 m2 [17.5 (30.6) m2], and by the distance from forest edge which ranged from 71 to 305 m [205

(54) m] (Fig. 2).

Table 2 Estimates of the capture probability for each sampling occasion, along with 95% confidence intervals, from CMR analysis (model Mt)

Capture probability estimates		
Occasion	Capture probability	95% Confidence Interval
1	0.03	0.00 – 0.10
2	0.10	0.04 – 0.23
3	0.12	0.05 – 0.27
4	0.05	0.01 – 0.14
5	0.03	0.00 – 0.10
6	0.15	0.06 – 0.31
7	0.03	0.00 – 0.10

4. Discussion

4.1. Sampling effectiveness and population density

In the present study we provided further evidence that the application of hierarchical models for addressing forest management plans is highly effective and requires a much lower research effort than other methods, as recently reported by Romano et al. (2017). Indeed, as a preliminary result, naïve occupancy data (i.e. ascertained presence during only three surveys) provided the same distribution pattern of salamanders shown by the more time-consuming research performed over seven days for CMR (when shelters were also searched for salamanders). Consequently, monitoring these salamanders by removing debris and searching under shelters (Bonato & Fracasso 2015), when the animals are not active due to non-optimal weather conditions, is disruptive on the habitat. Our salamander density estimates (39/ha, 95% C.I = 28-50, see Results), were found in an area which is the limit of the known distribution range of this taxon. Density of Golden Alpine Salamander from Venetian Prealps (i.e. the core area of this taxon) has been found to range from 95 to 475 individuals/ha (95% C.I = 97-120 and 291-659, respectively) in two environments: in the first Norway spruce is dominant and low rocky burrows available, while in the second, silver fir and beech are dominant and rocky shelters abundant (the second; Bonato & Fracasso 2003). Our estimates are lower but somewhat comparable with those obtained by Bonato & Fracasso (2003) from the less suitable environments in their study area. Local densities and population sizes of animals often decline from the core of their

ranges towards their distribution limits (Hengeveld & Haeck 1982; Brown 1984) although this trend should not be considered a general rule (see Gaston 2009 for an overview on this topic). The level of habitat occupancy also declines towards range limits, where a higher population fragmentation is expected (Gaston 2003; Yakimowski & Eckert 2007). However, the observed changes in density towards the population range limits may be stem from the spatial scale considered: they may be the resultant of changes in occupancy levels rather than actual variation in population densities (Gaston 2009). Therefore, the patterns of occupancy and density level we observed should also be considered from this perspective.

4.2. Site variables and ecological outcomes

Our results suggested that covariates that are often significant in explaining occurrence patterns in other forest salamanders, (e.g. topographic wetness index or other GIS derived, number of trees, amount of deadwood; see for example Otto et al. 2013; Romano et al. 2017), have a trivial effect on occupancy for the Alpine Golden salamander. In this case, the distance from the forest edge (EDGE) and the amount of brushwood piles (BRUSH) were much more relevant in explaining the observed occupancy pattern (Tab.1). Clearly, these two sets of variables act at two different spatial scales. The former acts on the general distribution of salamanders, while the second maximises habitat suitability in the range of tens or hundreds of square metres. Although the nominal subspecies of the Alpine Salamander, *S. atra atra*, also occurs in open and rocky habitats, *S. a. aurorae* is strictly associated to mature forest environments (Bonato et al. 2007). Indeed, we provided the evidence of the negative effect of the forest edge proximity on this taxon which is particularly sensitive to high level of moisture (Bonato & Fracasso 2015; Lefosse et al. 2016). Distance from clearcut or open pasture edges significantly affects the distribution of several amphibian species, especially that of the forest salamanders (deMaynadier & Hunter 1998). Considering the useful distance from the forest edge to provide suitable habitat conditions for the Golden Alpine Salamander (at least 200 m), forest management actions that reduce the forested area and increase fragmentation

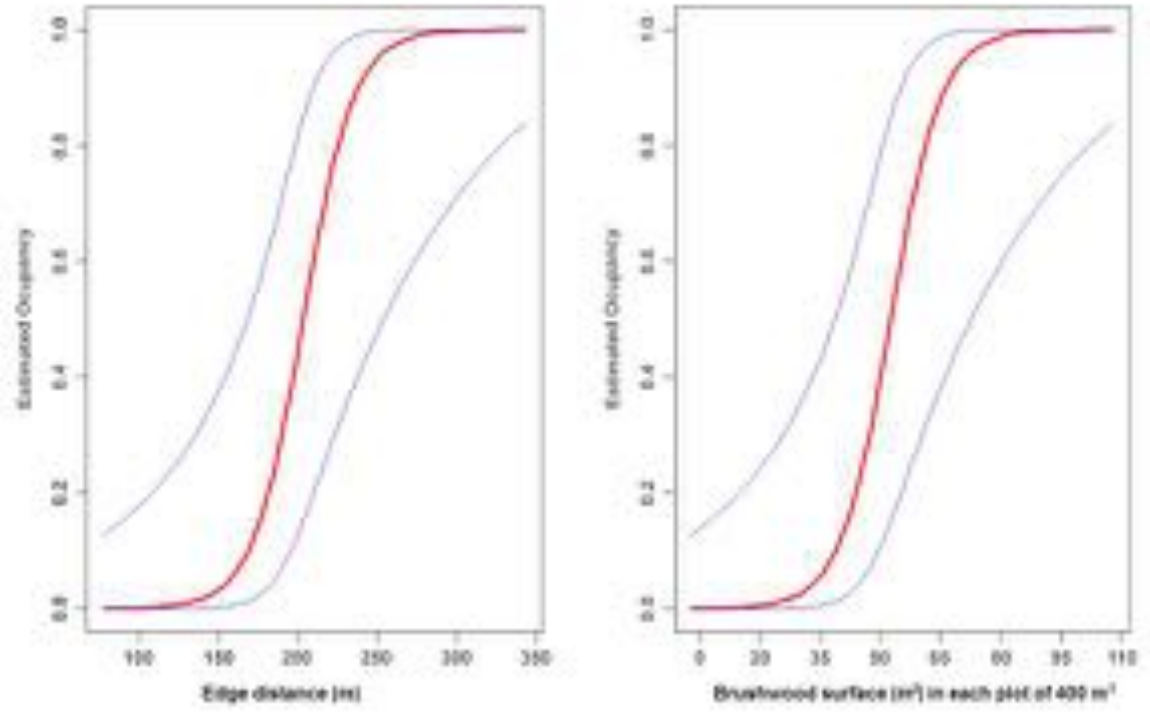

Figure 2 Effect of EDGE and BRUSH on occupancy (Mean and 95% CI), as predicted by the best model including these covariates.

have to be avoided for the conservation of this endangered salamander. At a smaller scale, a highly significant positive effect on the presence of the Golden Alpine Salamander was provided by the presence of brushwood piles. Deposits of woody debris are a multifunctional resource because within them amphibians may find both shelter and food (Indermaur et al. 2009). A It has been shown that recent cut woody debris may not be suitable for amphibians because small sizes or an insufficient decay stage do not provide adequate moist refugia (Petranka et al. 1994; Kluber et al. 2009; Davis et al. 2010). However, in such cases "dead wood" generally refers to coarse woody debris (CWD) which is defined in the literature in many ways (see Yan et al. 2006 for a review). The USDA Forest Service and Long Term Ecological Research (LTER) have provided a standardised definition and classification (Harmon & Sexton 1996; Harmon et al. 1999). The minimum diameter of CWD is ≥10 cm at its widest point while debris with a diameter from 1 to 10 cm should be defined as fine woody debris (FWD). Like many other small vertebrates (Fauteux et al. 2012), amphibians are known to benefit considerably from using coarse woody debris (CWD) as thermal, moist and feeding refugia (Davis et al. 2010 and references therein; Otto et al. 2013; Blomquist &

Hunter 2010). Conversely, the role played by FWD in vertebrate conservation ecology has been poorly investigated (e,g. Goszczyński et al. 2007; Indermaur & Schmidt 2011). The brushwood piles present in the plots of our study site may be classified as FWD. Soil moisture in brushwood piles exceeds soil moisture levels in many surrounding microhabitats, mitigating the negative desiccation effects of timber harvest (Rittenhouse 2007; Rittenhouse et al. 2008). This positive effect is intuitively particularly significant for taxa requiring high level of habitat moisture, such as the Golden Alpine Salamander. After harvest, total brushwood in a forest stand may be divided into several medium-sized piles or merged into one large pile. Ober & Minogue (2007) suggested that the biodiversity value of brushwood piles can be increased by creating several medium-sized piles (1.5-2.5 m x 3 m) rather than one large pile. In our study area within the plots hosting more than one salamander, there were generally several brushwood piles with > 3x2 m in size (ranging from about 0.5-1.5 m in height), which corresponds to the best situation suggested by Ober & Minogue (2007). Further studies could elucidate whether and what extent the decay stage of this FWD can also play a role in maximising the habitat suitability of the plots for *Salamandra atra aurorae* and other vertebrate taxa. FWD potentially represents a considerable portion of the total volume of dead wood in temperate forests, especially in managed ones (Nordén et al. 2004). Traditional silvicultural harvest methods produce large quantities of FWD of little commercial value. Therefore, retaining brushwood piles in managed forests may contribute to biodiversity conservation with no economic conflict. In this respect, possible future policies to increase the share of energy produced with biomass should strongly consider potential impacts on biodiversity conservation.

4.3. Conclusions

Ongoing studies will ascertain whether N-mixture models can be used to assess population trends (i.e. variation in abundance) for this salamander, which is a particularly difficult task to achieve for secretive and locally rare species (Ficetola et al. 2017; Ward et al. 2017). For conservation purposes, as stipulated by the regional administration of Veneto (Art. 232. Annex A, DGR no. 786/2016) which hosts about 90% of the current known distribution range of *S. atra aurorae*, and the Forest Management Plans of the municipality of Levico Terme (Trentino region), forest harvesting can only be carried out from mid-October to the end of April, preferably in ice-covered or snow-covered ground

conditions, selecting logging machineries known to cause the lowest possible soil compaction and minimising the transit of vehicles outside roads and permanent trails. Our results suggest that conservation plans for the Golden Alpine Salamander should consider its high sensitivity to the "forest edge" effect. In addition, provision could be made under existing municipal and/or regional legislation to ensure small-scale habitat improvement by leaving FWD organised in several brushwood piles within the forest, which is a simple and sustainable action due to the low commercial e value of brushwood.

Aknowledgments

Experimental protocols were approved by the Italian Ministry of Environment (authorisation PNM-EU-2017-005370). This study was supported by the Provincia Autonoma di Trento (PAT), by "Servizio Aree Protette e Sviluppo Sostenibile" (Convention PAT LIFE TEN) and by MUSE. Thanks are due to Dr. Giorgio Zattoni, Davide Sartori and the staff of the Corpo Forestale Trentino who greatly contributed to the effectiveness of our research, providing logistic facilities close to the sampling site and making useful suggestions. Nicola Gozzer and Fabrizio Iori (forest wardens of the municipality of Levico Terme) kindly contributed to the data collection of environmental variables. Matteo Anderle, Matteo Facchinelli, Riccardo Novaga, Costanza Piccoli, Marianna Potrich and Chiara Trevisin (MUSE staff and volunteers), greatly contributed to field sampling. Aaron Iemma and Simone Tenan (Section Vertebrate Zoology, MUSE) provided useful suggestions and stimulating discussions. "Servizio Foreste e Fauna", Elisabetta Romagnoni and Matteo Sartori (PAT) enthusiastically supported this project and provided useful information. Mark Walters provided linguistic revision. We also thank two anonymous Reviewers, whose comments and suggestions greatly improved a previous version of the manuscript. There are no conflicts of interest associated with this publication and there was no significant financial support for this work that could have influenced its outcome.

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Chapter 3

Conservation of salamanders in managed forests: methods and costs of monitoring abundance and habitat selection

Part of this chapter is published in: Romano, A., Costa, A., Basile, M., Raimondi, R., Posillico, M., Scinti Roger, D., Crisci, A., Piraccini, R., Raia, P., Matteucci, G., De Cinti, B. (2017). Conservation of salamanders in managed forests: methods and costs of monitoring abundance and habitat selection. *Forest Ecology and Management* 400: 12-18.

Abstract

Ecological traits affect species' responses to human impacts. Amphibians are declining worldwide and one of the major causes of such decline is habitat loss. Forestry practices have a primary role in determining habitat loss and fragmentation for amphibians. Thus, researchers should provide forest practitioners with essential information in order to address proper forest management plans. Here, we studied ecological requirements in the terrestrial phase of salamanders and we tested the feasibility of repeated counts to infer habitat determinants of salamanders' abundance in order to guide forest management plans. We employed the N-mixture models for the analysis of repeated count data of an Italian endemic salamander (*Salamandrina perspicillata*) on seventy-seven 100 m^2 plots, on a central-Italian forest. Modelling salamanders' abundance as a function of site specific habitat features allowed us to give precise guidelines for forest management. Harvesting should be conducted on south facing slopes, since salamanders' occurrence and abundance are higher on northern slopes. Forest operation should be avoided within a buffer of some 150 m from reproductive sites. Since salamanders use tree bases as shelters, patches of forest with larger trees and higher canopy cover should be retained, ensuring the availability of moist shelters. The amount of the cost for the whole monitoring, from plot installation to data analyses was 4,872 Euros (about 5,558 USD). Given the ease of application and inexpensiveness of this sampling protocol, we encourage its employment in similar situations, in order to gather useful information, which are essential to couple forestry practices with species conservation strategies.

1. Introduction

Amphibians populations are declining worldwide and the increasing extinction rate in this vertebrate class is exceedingly high (Stuart et al. 2004; Wells 2007). Among the many underlying causes of amphibians' decline, habitat loss and fragmentation are supposed to give a strong contribution (Wells 2007). The physiological and morphological traits of amphibians and the strong dependence from the water for biphasic species, make them highly reliant on narrow ecological conditions and heavily exposed to microhabitat alteration (Welsh 1990; deMaynadier & Hunter 1995; Grover 1998, 2000; Peterman & Semlitsch 2013). Among the anthropogenic activities resulting in habitat loss, fragmentation and alteration, forestry practices may alter the habitat as a whole (Carlson & Groot 1997) or in its microhabitat features (Riffel et al. 2011). The link between amphibians ecology, conservation and forest productivity has been well-studied and intrigued ecologists and conservation practitioners for years (deManyader & Hunter 1995). For instance, it has been observed how intensive forestry (*e.g.* clearcutting) can reduce abundance and alter the survival of amphibian populations (deManyader & Hunter 1995; Semlitsch et al. 2009). Moreover, some forestry practices, such as deadwood removal, can reduce amphibians' abundance and survival as well through the reduction of shelters and foraging sites (Otto et al. 2013). Also, among the main negative consequences of forest harvesting on small vertebrates, the risk of crushing during logging operations may be responsible for more than half of the deaths in a given population (Penman et al. 2005; Escobar et al. 2015). The seasonal scale of forestry practices should also be considered. Indeed, even if amphibians are considered one of the most sedentary *taxa* among vertebrates, they perform large seasonal movements, being reliant to large portions of the surrounding habitat during a limited period (see Wells 2007 and references therein). Many biphasic amphibians exhibit some sort of seasonal, migration-like, movement from wintering/terrestrial shelters to reproductive sites and vice-versa (Wells 2007). Then, accordingly to modern conservation approaches, preserving amphibian populations requires the protection and management of terrestrial habitat and not merely the aquatic breeding sites. The surroundings of the breeding pools were considered as priority areas for the maintenance of amphibian populations in North America (Welsch et al. 1995; Semlitsch 1998). However, information on the size and shape of these terrestrial buffers is very heterogeneous, spanning from about 15 to 120 m around the pools (New

Hampshire Division of Forests and Lands, 1997; Calhoun & deMaynadier 2002, respectively). For these reasons, reliable inferences on the ecology and local abundance of amphibian populations are an essential tool in planning forest management practices in a sustainable approach. In the present study, we used and tested the feasibility of N-mixture models (Royle, 2004) to infer habitat determinants of salamanders' abundance in order to guide forest management plans. We performed our study in an Italian forest, using Northern spectacled salamander, *Salamandrina perspicillata* (Savi 1821), as target species. The aim of this study was threefold at ecological, conservation and management, and economic levels. First, we investigated the ecological traits related to the fine scale habitat selection of salamanders in a forest stand, by estimating local abundance in relation to environmental features. Second, as a result of the first objective, we suggested reliable information for conservation planning in relation to forestry. Finally, we evaluated the costs of both sampling and analytical framework, with particular attention to the obtained results and the best management guidelines consistent with the outcomes of our analysis.

2. Materials and methods

2.1 Study species

Salamandrina perspicillata is endemic to northern and central Italy (Romano et al. 2009). This species is a small sized biphasic salamander, inhabiting mainly shady and damp areas but also Mediterranean habitats. Adults are terrestrial and only females go to water just for spawning (Lanza et al. 1983; Angelini et al. 2007). This species shows a quasi-bimodal activity pattern during the year, with two peaks of activity in Spring and Autumn (authors pers. obs.). Many studies provide information about the reproductive biology of *Salamandrina perspicillata*, however terrestrial ecology of this forest-dwelling salamanders is almost unknown (cf. Angelini et al. 2007).

2.2 Study area, study framework and sampling design

The study area is an Apennine forest stand located in Central Italy (41°44'50 N, 14°11'40'' E), -included in the Collemeluccio-Montedimezzo UNESCO-MAB Biosphere Reserve, at about 900 m a.s.l.. The vegetation is best categorized as a Supra-Mediterranean mixed deciduous forest (Blondel and Aronson 1999), dominated by beech (*Fagus sylvatica*) and Turkey oak (*Quercus cerris*). We identified, delimited, GPS-positioned and individually marked 77 square plots, each one measuring 100 m^2 (Mean distance between plots ± SE; 27.7±0.75) (Fig.1). The study area includes

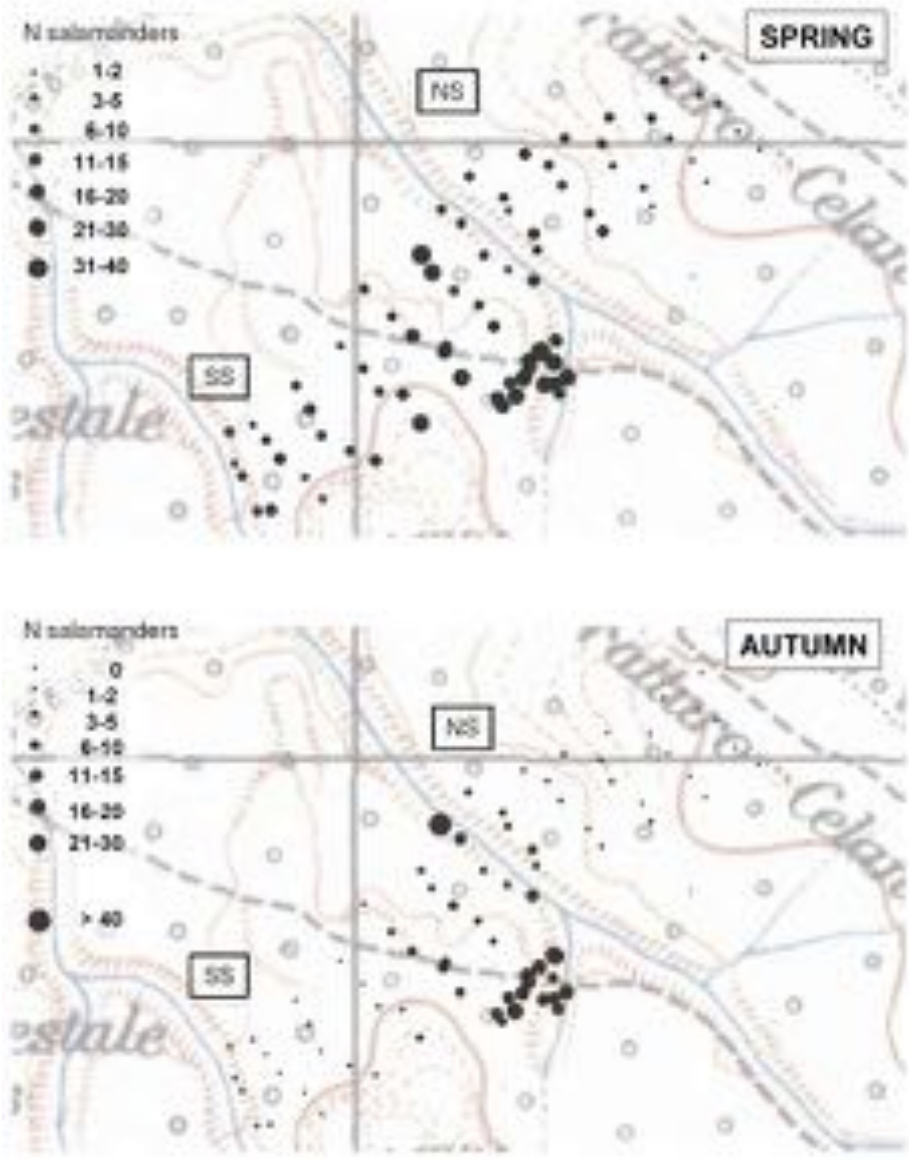

Figure 1 Map representing the spatial distribution of sampling sites and the estimated abundances interval (density on 100 square meters) of salamanders during Spring and Autumn. NS (Norther stream) and SS (Southern stream) are the two breeding sites.

a northern stream (NS) and a southern stream (SS), which run in parallel at a straight-line distance of about 350 meters. A drainage divide separates the streams, 100 m higher in elevation than both bed streams. Streams flow at about the same elevation with a southeast to northwest direction. Thirty-six plots overlapped the nodes of a virtual grid with a mesh of 30x50 meters between NS and SS, where the forest is dominated by beech (Fig.1; 30 m of distance among plots parallel to the streams and 50 m among plots perpendicularly to the streams). Fifteen additional plots were randomly placed at about 5 to 100 meters from a secondary NS stream branch, on a southeast slope, in a mixed deciduous portion of the forest. Finally, 26 plots were placed, using

the above-mentioned grid, on the right slope of the NS, in a Turkey oak dominated forest (Fig.1). Both streams (NS and SS) are breeding sites for *Salamandrina*, although NS is the main breeding site for salamanders of the whole natural reserve while the smaller streams (*i.e.* SS) may be considered as secondary breeding sites (authors pers. obs.). Thus, we had a total sampling area of 0.77 ha, included within 11 ha of forest stand. We employed the 77 plots as sites for our spatially and temporally replicated surveys. During the Spring (late April 2014) and the Autumn (early October 2014), all sites were visited three times by the same observers (4 persons team, consisting of an expert herpetologist and three M.Sc students). During each survey the observer sampled a site for four minutes before moving to the next sampling site, looking on the leaf litter, checking trees' buttresses and cavities with electric torches and recording the total number of salamanders per plot. Each sampling session lasted about two hours. All samplings were carried out during favourable climatic conditions (*i.e.* wet or slight rain and under limited winds) in order to maximize the probability of detecting active salamanders.

2.3 Data analysis

We measured six environmental variables, describing salamanders' habitat for each plot. We recorded the number of trees (TREES) with a diameter at breast height larger than 10 cm, since this variable gives information on both forest structure (Weller 1987) and availability of shelters for salamanders (Piraccini et al. 2016, Basile et al. 2017). Five more variables were GIS-derived. We calculated the distance of each plot from the nearest reproductive stream (DIST); then, using a digital elevation model (30 m cell), we calculated the aspect of each cell, and its northness (ASPECT) was calculated as the cosine of the aspect angle, ranging between -1 (southward aspect) and 1 (northward aspect), and assuming values near zero for cells facing east or west. Moreover, we calculated the topographic wetness index (TWI; Sorensen et al. 2006; Peterman & Semlitsch 2013), which is a measure of surface wetness for each grid cell, and the total insolation (INSOLATION; Tovar-Pescador et al. 2006) at land surface, expressed as Kw/h per square meter. Finally, we calculated the normalized difference vegetation index (NDVI; 30 m cell; Peterman & Semlitsch 2013) for Spring and Autumn, using Landsat 8 multispectral images, which underwent through an atmospheric correction procedure. The NDVI is highly correlated with the Leaf Area Index and the net primary productivity (Myneni et al. 1995; Pettorelli et al. 2005; Lee et al. 2004).

Approaching data analysis, as a first step, we conducted a correlation analysis between site covariates in order to identify possible collinearity (MacNally 2002). Consequently, repeated count data were analysed using N-mixture models for closed population (Royle 2004). We assumed population closure since our surveys lasted few days and Spring and Autumn data were analysed separately (Peterman & Semlitsch 2013). N-mixture models provide estimates of two parameters: the state variable, *i.e.* mean abundance of salamanders per site (*n*), and the probability of detection (*p*) (Royle 2004; Kéry & Royle 2015). The total abundance of salamanders (*N-hat*) and, being known the surveyed area, the density (*d*) can be estimated as derived parameters. To model abundance, we built several global models (*i.e.* the most complex models, in which other candidate models are nested) using different distributions (*i.e.* Negative Binomial; Poisson; Zero-Inflated Poisson) and checking for parameter estimate stability, while using different values of the upper limit of integration (*K*). Then all global models were tested for goodness of fit by mean of a Pearson chi-square test (MacKenzie & Bailey 2004), using a parametric bootstrap resampling (1000 resampling). P-value of the test and overdispersion parameter (*c-hat*) were used to select the best model to be employed as the global one (Burnham & Anderson 2002). Starting from the best global model we built all possible nested models deriving from covariates combinations and detection probability structures: salamanders' abundance (*n*) was modelled as a function of site covariates, while detection probability (*p*) was considered both constant over time or survey-dependent. Models were selected according to second-order quasi-likelihood Akaike's Information Criterion (*i.e.* QAICc, Akaike 1973; Burnham & Anderson 2002), considering that models with a ΔQAICc > 2 show substantial performance differences (Burnham & Anderson 2002). When more models were laying within a ΔQAICc < 2 interval, then model averaging techniques, based on QAICc weights, were employed to obtain averaged estimates (Burnham & Anderson 2002). All GIS analyses were conducted with software SAGA GIS (ver. 2.1.2), while model building was conducted in R environment with "unmarked" (Fiske & Chandler 2011) and "AICcmodavg" (Mazerolle 2011) packages.

Finally, we provided a detailed estimate of costs for the application of this sampling protocol. To obtain this costs-list we tallied all the expenditures required for conducting the study in two seasons and in a forest stand of similar extension. We considered both real costs such as researchers' remuneration, field equipments or logistical requirements, and the survey and data analysis costs, calculated as hours or days of

sampling/working effort. The estimates of costs regarding the equipment were obtained by the mean of mid-range equipments' costs from international online sellers, while the expenditure for researchers' retribution and accommodation has been calculated on the base of the average Italian values.

3. Results

During the surveys, we counted a total of 449 salamanders (290 in Spring, 158 in Autumn). Spearman's rank correlation coefficient for site covariates indicated lack of significant correlation ($\rho_s < 0.7$; $P > 0.05$; N=77), allowing us to use all the covariates in model building procedure. Among the global models candidate set, the model with negative binomial distribution and $K = 50$ was the one with the best fit and lower overdispersion, both in Spring ($P = 0.22$; *c-hat* = 1.08) and Autumn ($P = 0.16$; *c-hat* = 1.43). Therefore, 114 nested models resulted from the global model, for each season. Model selection returned one model in a ΔQAICc ≤ 2 range for Spring (Table 1); while in autumn three models showed a difference of less than two points of QAICc (Table 1). Regarding the detection probability structure, all the best models in both seasons, accounted for survey-dependent detectability. During the Spring, the abundance of salamanders is positively

Table 1
List of candidate models laying within a ΔQAICc < 2 range.

Spring			
Model	Par. n.	QAICc	ΔQAICc
p(.) λ(.exp + times + twi)	9	496.59	0.00
Autumn			
Model	Par. n.	QAICc	ΔQAICc
p(.) λ(.exp + ndvi)	8	356.03	0.00
p(.) λ(.exp + insolation + ndvi)	9	356.90	0.87
p(.) λ(.exp + twi + ndvi)	9	357.20	1.22

Table 2
Averaged beta estimates for spring.

Spring – Beta estimates			
Parameter	Estimate	SE	95% Confidence limits
Intercept	0.86	0.21	0.46–1.27
Aspect	0.97	0.11	0.76–1.19
Times	−0.68	0.15	−0.97 to −0.40
TWI	0.58	0.09	0.41–0.75

Table 3
Averaged beta estimates for autumn.

Autumn – Beta estimates			
Parameter	Estimate	SE	95% Confidence limits
Intercept	2.21	0.34	1.55–2.88
Aspect	0.65	0.16	0.34–0.96
NDVI	0.46	0.24	−0.00–0.92
Insolation	0.22	0.17	−0.11–0.55
TWI	0.15	0.12	−0.10–0.39

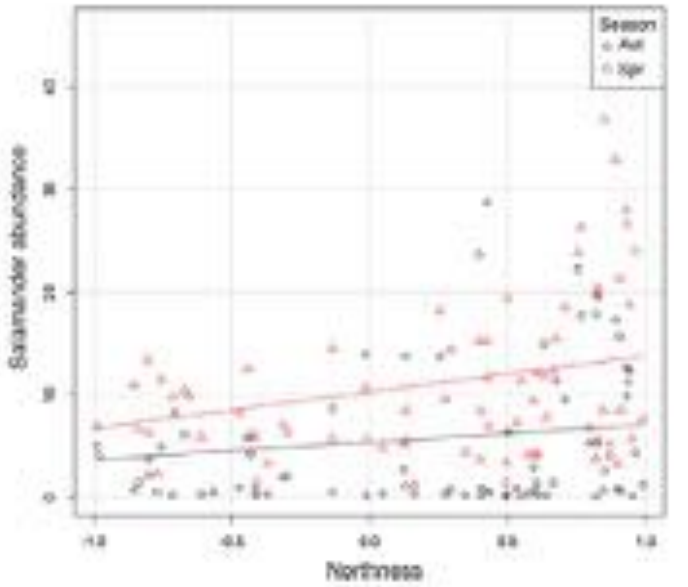

Fig. 2. Scatterplot representing the relationship between northness and estimated salamanders' abundance.

related to higher values of ASPECT (northward cells) and TWI, while TREES has a negative effect on *n* (Table 2). In Autumn, where three models lie within a ΔQAICc $\leq$ 2 range, the variables' ranking, based on their importance (*W*t; Mazerolle et al. 2005), showed that ASPECT and NDVI are the most important ones in describing salamanders' abundance (W*t* = 1.00, for both variables), followed by INSOLATION (W*t* = 0.30) and TWI (W*t* = 0.25). Furthermore, in this season all variables have a positive relation with salamanders abundance; averaged Beta estimates of abundace covariates, togheter with their 95% confidence interval (CI) are presented in Table 3. The variable ASPECT, besides being the most important one, also had the strongest effect on describing salamanders' abundance (Beta estimate = 1.56; 95% CI, 1.02–2.09; Fig.2), highlighting higher salamanders abundance on northward slopes. We obtained a mean abundance per site of 9.85 salamanders in Spring (95% CI, 7.5–13.2) and 11.23 in Autumn (95% CI, 4.21 – 21.35). The density calculated for the sampled area is 575 (95% CI, 301–691) and 1,135 (95% CI, 270–1,429) salamanders/ha, in Spring and Autumn, respectively. Finally, the amount of the cost for plot installation and monitoring, data analysis and equipments was 4,872 Euros (about 5,558 USD). The detailed list of expenditure items is provided in Table 4.

4. Discussion

The results we present here showed that local abundances of *Salamandrina* are driven by different variables depending on the season. The variable ASPECT, *i.e.* northness of the slope, is the most important variable describing salamanders abundance both in Spring and Autumn, highlighting higher abundances on north-facing slopes (Fig.2). Field observations suggested that Spectacled salamanders inhabits - at a coarse scale - cool, shady and damp valleys (Barbieri & Pellegrini 2006) but further studies showed that it may occur in drier environments as well, such as the Mediterranean maquis (*e.g.* Angelini et al. 2008; Romano et al. 2007; Romano & Ficetola 2010). In contrast, small-scale data are lacking. ASPECT is commonly known to have a dominant role in determining the microhabitat and ecological conditions of sites: indeed slope of a surface affects the amount of incoming solar radiation, which in turn affects surface temperatures and water evaporation (Geiger 1965; Oke 1987). For instance, temperature variations occurring within adjacent slopes can resemble those occurring between sites differing by 5° of latitude or 500 m of altitude (Bennie et al. 2008), having a main role in determining vegetation structure, species occurrence and diversity (*e.g.* Boyko 1947; Badano et al.

1. PLOT INSTALLATION AND MONITORING

Field effort

Description	Generalized effort		Effort in our study	
	Man-hours	Man-sampling days	Hours (4 persons team)	Sampling days (4 persons team)
Identification of plots, installation, GPS positioning and field covariates measurements	12	2	3	1
1 survey of 77 plots (4 min per plot + 1 min for movement between plots)	7	1	2	1
6 surveys of 77 plots	42	6	12	6
Total	54	8	15	7

Economic costs

Description	Generalized costs		Costs in our study	
	Man-sampling days	Cost/person * day^{-1}	Sampling days (4 persons team = 1 researcher and 3 students)	Cost/person * day^{-1}
Remuneration for research officers conducting field surveys	8	228EUR (260USD)	7	228EUR (260USD)
Total	1824EUR (2080USD)		1596EUR (1820USD)	

2. DATA ANALYSIS

Effort and costs

Description	Man-hours (1 researcher)	Remuneration costs
Data computerization	2	228EUR (260USD)/person * day^{-1}
GIS-derived covariates calculation	14 (2 working days)	
Data analysis with N-mixture models	28 (4 working days)	
Total	44 (7 working days)	1596EUR (1820USD)

3. ADDITIONAL RUNNING AND EQUIPMENT COSTS

Description	Generalized costs		Costs in our study (4 persons team)	
GPS device	200EUR (228 USD)		-	
Mid-range personal computer (8 GB ram and quad-core CPU)	600EUR (685 USD)		-	
Software and GIS layers	Open source softwares and free GIS layers		Open source softwares and free GIS layers	
Accommodation and food during surveys	60EUR/man * day^{-1} (68USD/man * day^{-1})		60EUR/man * day^{-1} (68USD/man * day^{-1})	
Total	1280EUR (1462USD)	Study equipment and accomodation for one observer for 8 sampling days	1680EUR (1918USD)	Study equipment and accomodation for 4 observers for 7 sampling days

4. TOTALS

Description	Generalized total	Total in our study
Working days for the complete study (installation, surveys and data analysis)	15	14
Costs for the complete study (installation, surveys and data analysis)	4700EUR (5326USD)	4872EUR (5558USD)

Table 4 List of costs and time employed for the complete study. Expenditures and survey efforts detailed for our study and genealized costs and effort for a single researcher.

2005). Regarding amphibians, and in particular lungless salamanders and those with vestigial lungs (*i.e. Salamandrina*) mainly relying on skin for gas exchange, the effect of soil moisture and relative humidity is thus a key factor influencing their occurrence and activity (Wells 2007; Peterman & Semlitsch 2013; Costa et al., 2016). The availability of water and soil moisture is also described by TWI, which has been related both to vegetation structure and habitat selection on salamanders (*e.g.* Kopecký & Čížková 2010; Peterman & Semlitsch 2013), highlighting a positive relationship with the local abundance of *Salamandrina*, especially during Spring. During this season, salamanders abundance is also negatively affected by TREE (*i.e.* tree density). This result depends on the fact that salamanders use burrows and cavities near the buttresses of larger trees (Piraccini et al., 2016) whose number is inversely correlated to tree density (Weller 1987; Lonsdale 1990; Spiecker et al. 2009). Moreover,

salamanders in Spring are generally more abundant near the main reproductive site (NS), where tree density is lower. In Autumn, NDVI has a main role in describing salamanders' density. Indeed, higher estimates of salamanders' abundance are related to higher values of NDVI. This variable is mainly related to canopy cover, water availability and net primary productivity (Pettorelli et al. 2011; Peterman & Semlitsch 2013), and may indicate multiple effects on salamander abundance. Higher canopy cover can prevent soil desiccation and provides higher levels of moisture, which can be directly linked both to shelter availability and its suitability. Indeed, closed canopy can deliver a higher amount of water at the ground level around trees, via stem flow (Gersper & Holowaychuk 1971; Jordan 1978), determining wetter shelters typically used by *Salamandrina*. During Autumn, in sites characterized by wet and cool environments, like the study area (mean annual temperature is 8.6 °C and the mean annual rainfall is 1,022 mm; Calamini et al. 2011), salamanders select environmental patches with a higher NDVI, reflecting the presence of shaded patches, while the TWI was quite homogeneous over the study area. In this season, INSOLATION was not significant, as temperature decreases and Winter approaches, and salamanders tend to disappear and retreat in underground shelters (Angelini et al. 2007). Regarding the link between salamanders' ecology, their habitat selection and forestry practices, we believe that the outcomes of the present study provide important information for planning forestry treatments, with the aim of maintaining high levels of habitat suitability for Salamanders. Indeed, since addressing forestry operations is a key factor for minimizing habitat alteration and maintaining viable populations, after a deep knowledge of habitat use and selection in different seasons we can give practical guidelines regarding our case-study. For instance, given the strong effect of ASPECT in both sampling seasons, underlining higher abundances on north facing slopes, we suggest that forestry operations should be conducted on south facing slopes, avoiding or limiting those on the northern ones. Moreover, we suggest that a minimum buffer zone of about 150 meters (but possibly more) from streams used for spawning should be retained in Autumn (Fig.1), in order to preserve integrity of habitat and shelter features near reproductive sites. In Spring, during the post breeding season, salamanders were distributed more homogeneously in the area (Fig.1) and to suggest a minimum buffer zone was not feasible. Finally, given the negative effect of TREE density, and its link with the availability of larger trees used as shelters, we suggest that harvesting interventions

should be planned with the aim of retaining larger trees and those providing higher levels of canopy cover (as suggested by NDVI), which in turn can prevent soil desiccation and provide higher levels of moisture (as suggested by TWI). Different salamander species may require different level of moisture and canopy cover and they may move at different distances from their breeding sites (e.g. Wells, 2007). Here we provided detailed information for *Salamandrina* but general consideration and the study approach may be generalised fro many fully terrestrial or semi-terrestrial salamanders. The last aim of our study regarded the effectiveness of applying hierarchical models for addressing forest management plans. The field activity of our study required only seven working days to a four persons team (Tab.4). Moreover, even though a single researcher, given the simplicity and ease of the sampling protocol, conducted the study it would require only eight days of field sampling. Furthermore, since the field operations of this sampling protocol do not require specialized skills, remuneration costs can be cut down employing students and volunteers (as in our study). Actually, volunteers may provide huge amount of data at low cost, and volunteer-based methods may be highly effective to detect the trends of species, particularly of those living in easily accessible areas (e.g. Kéry et al. 2009; Griffiths et al. 2015). Intuitively, costs of sampling may vary greatly from region to region. Regarding data analysis, the complete process including data computerization, GIS covariate calculation and N-mixture model building required only seven days of work for a researcher. Obtaining quantitative measures of population trends or of variations in abundance or species assemblage before and after an habitat disturbance (e.g. a forest treatment) is often challenging, as it often requires repeated surveys and high field efforts (e.g. Mitchell et al. 1997; Smith & Sutherland 2014). The use of species inventory to evaluate the effects of habitat disturbance is a sensible and applicable approach only when the number of species is high, such as for herpetofauna assemblage in American or Asian environments (Hutchens & De Perno 2009; Sung et al. 2012). Conversely, when the number of indicator species is low (as for amphibians in European countries) the species assemblage is not informative and parameters at population level have to be analysed. Therefore, taking into account the information obtained from the present study for addressing forest management plans, together with the easy application and the relative cheapness of this sampling framework, we suggest that the adoption of a similar sampling protocol could be employed in order to gather information

concerning forestry practices and species conservation strategies.

Acknowledgments

This study was supported by the Life project ManFor CBD LIFE09 ENV/IT/000078. Experimental protocols were approved by the Italian Ministry of Environment (authorisation PNM-II-2012-0015691). Rodolfo Bucci and Filippo La Civita contributed to field sampling. Tiziana Altea (Ufficio Territoriale per la Biodiversità Castel di Sangro – Corpo Forestale dello Stato), Domenico De Vincenzi (Ufficio Territoriale per la Biodiversità Isernia – Corpo Forestale dello Stato) and the Posto Fisso di Montedimezzo personnel greatly contributed providing logistic facilities. There are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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PART TWO – N-mixture models in amphibian population monitoring

Chapter 4

Estimating abundance of the Stripeless tree-frog *Hyla meridionalis* by means of replicated call counts

Part of this chapter is published in: Crovetto, F., Salvidio, S., Costa, A. (2019). Estimating abundance of the Stripeless tree-frog Hyla meridionalis by means of replicated call counts. *Acta Herpetologica* 14, 71-75.

Abstract

The Stripeless tree-frog *Hyla meridionalis* reaches its eastern-most European distributional limit in NW Italy, and specifically in the Cinque Terre National Park. Here for two consecutive years, we estimated tree-frog population abundance by call surveys at 24 sites. Data were analysed in the framework of N-mixture open population models based on repeated counts of calling males. The results obtained by this statistical approach were effective in estimating population size together with annual recruitment and survival. The tree-frog male population size remained constant between years and site abundance was inversely related with altitude. On the bases of these findings, our application of N-mixture models to tree-frog calling males was successful and is a promising cost-effective method to obtain long-term monitoring data on this species over large geographic areas.

1. Introduction

The Stripeless tree-frog *Hyla meridionalis* Boettger, 1874 is found in North-western Africa (Algeria, Morocco and Tunisa), South-western Europe (Portugal, Spain, S France and NW Italy) and the Canary and Balearic Islands (Sillero, 2010). On a portion of its distribution range the species is considered introduced, i.e. Canary and Balearic Islands (Sillero et al., 2014), and it is also possibly introduced for other European regions (Recuero et al., 2007). In Italy, the Stripeless tree-frog is common along the Mediterranean coast of Liguria (NW Italy), from the Province of Imperia to the province of La Spezia (Salvidio, 2007). Apart from morphometric and distributional data (Salvidio, 2007), little is known about the abundance and dynamics of Stripeless tree-frog populations in Italy, and quantitative data on populations size should be obtained to assess the species status and its ecological requirements in particular near the species distribution limits, where a high population fragmentation is expected (Gaston, 2003). Although photo-identification of Stripeless tree-frogs is possible (Crovetto unpublished data), the animals are arboreal and highly secretive during daytime. The use of PVC pipes may increase the probability of detection of tree-frogs (do Vale et al., 2018), however, in the CTNP the majority of the species' reproductive habitats are on private lands, and thus are not freely accessible (Romano et al., 2014). Therefore, the monitoring technique selected to estimate population size was based on nocturnal auditory surveys of calling males, because of the species highly distinctive mating call (Schneider, 1974; Márquez et al., 2005). Call survey is a relatively efficient technique for evaluating the distribution and diversity of anurans (Dorcas et al., 2009). Therefore, calling surveys are frequently used in large-scale amphibian monitoring programmes (e,g, Anthony, 2002; Weir and Mossman, 2005; Weir et al., 2005, 2009). However, the use of call surveys for estimating population abundances and trends suffers of the same problematic issues recognized in the case of repeated counts of individuals, because the detectability of anuran calling males is < 1 (i.e. not all males are calling in the same night, Pellet and Schmidt, 2005). Moreover, anuran mating call activities display high variation in response to biotic and abiotic factors, that usually remain unknown and difficult to model (Royle and Link, 2005; Droege and Eagle, 2009). In fact, using raw counts of calling males or even scores derived from abundance indexes (i.e. indexes that group calling males by classes of relative abundance; Weir and Mossman, 2005) without accounting for detection probability may lead to relevant bias in abundance and trend estimates (Schmidt,

2004; Mazerolle et al., 2007). Therefore, to reliably estimate population abundance, the information derived from raw counts of calling males should always be corrected for species-specific detection probabilities (Pellet and Schmidt, 2005; Royle and Link, 2005). Recently, specific modelling approaches have been proposed for estimating anuran population abundances from the count of anuran calling indexes taking into account detection probabilities (Royle, 2004a; Royle and Link, 2005). This study aimed to estimate the abundance of Stripeless tree-frog males together with some demographic parameters and ecological requirements in Italy, at the eastern limit of the species distribution. Moreover, we tried to establish a cost-effective monitoring protocol to provide future population trends. Because of the relatively small number of tree-frog males recorded per site, we had the opportunity to apply the open population generalization of Royle's (2004b) N-mixture model (Dail and Madsen, 2011) to count data derived from call surveys.

2. Materials and methods

2.1 Study area, study framework and sampling design

The eastern-most limit of the species' range in Europe is the village of Riomaggiore (Province of La Spezia), in the Cinque Terre National Park (CTNP), a protected area where the Stripeless tree-frog reproduces in streams and in artificial water tanks used for irrigation (Salvidio, 2007; Romano et al., 2014). In this area water streams display short and steep courses, with relatively long summer drying periods, due to the lack of precipitations (Olivari et al., 2013). Among many possible land use of rural areas, agriculture is the only one in the CTNP and from the sea level up to the hill tops vineyards and orchards are cultivated on strips of arable land, or "terraces", sustained by dry-stone walls. Irrigation is provided by means of water stored in tanks, often colonised by amphibians (Olivari et al., 2013; Romano et al., 2014). The survey sites were selected during both daytime and nocturnal preliminary surveys. During the day, streams and water reservoirs were inspected and selected as potential reproductive sites if adults, larvae or eggs of some amphibian species were observed. During the night, sites were located by perceiving the calls of Stripeless three-frog males. In total 24 sites were surveyed in the municipalities of Levanto, Monterosso and Riomaggiore (from West to East): 6 streams and 18 artificial water reservoirs in agricultural lands or urban settings (Table 1; Supplementary Materials Figure A1).

All surveys began after sundown and after hearing the first tree-frog calls. In 2017 three nocturnal surveys were performed, from the

Table 1. Continuous variables included in the N-mixture open population models (Dail and Madsen, 2011) used to estimate *Hyla meridionalis* abundance, in the Cinque Terre National Park.

Variable	Description	Sample size (N)	Mean (SD)	min	max
ELEV	Site altitude (m)	24	33.04 (29.66)	8	83
TEMP	Air temperature (°C)	7	17.14 (3.44)	12	21
WIND	Wind speed (m/s)	7	3.57 (1.27)	2	5
RH	Relative humidity (%)	7	66 (13.51)	45	85

end of March to May, by two operators that counted the number of males calling at each site during a two minutes period. In 2018, three nocturnal surveys were performed, from the beginning of May to the beginning of June, with the same observers and procedure of 2017. In addition, in 2018 a fourth survey was performed by a single operator that tallied calling males for 4 minutes. The asynchrony and the different tonalities of calls permitted to count with confidence the minimum number of males per site that, in all cases, was ≤ 6 (Supplementary Material Table A1). All sites were surveyed during the same overcast or rainy night, but never during heavy showers that could hinder a clear hearing of frog calls. Four climatic variables were obtained from the meteorological station of Levanto: rainfall during the 24 h preceding the survey (rain), air temperature (temp), relative humidity (rh) and wind speed (wind), recorded during the last hour of survey. These weather variables were selected, because they are known to influence anuran calling behaviour (e.g., Walls et al., 2011). Finally, for each site three variables were considered: altitude above the sea level (elev), a categorical variable for the municipality of the site (city) and if the water body was a stream or an artificial site (site).

2.2 Data analysis

Repeated count data were analysed using the Dail-Madsen (2011) model, which is a generalization of the Royle's (2004b) N-mixture model, capable of relaxing the closure assumption by considering the population closed to immigration/births and emigration/deaths during a short period (i.e. three/four survey nights performed each year), while considering the population demographically open between years, in a robust design-similar approach. This model estimates four parameters, two of which are in common with the Royle's (2004) N-mixture original formulation: individual detection probability (p) and mean initial abundance for each site (λ). The Dail-Madsen (2001) model estimates two additional parameters: the recruitment rate (γ), comprehensive of births and immigrations, and the apparent survival probability (ω), comprehensive of deaths and emigrations. In our study, we built models with Poisson error distribution, since Negative Binomial distribution could lead to identifiability issues and may produce infinite abundance estimates (Barker et al.,

2017; Link et al., 2018). Furthermore, in order to avoid truncated estimates of abundance (Knape et al., 2018), we set the upper limit for integration (K) to 50 (i.e. we checked estimate stability at incremental values of K). We then began the model building procedure by fitting a global model (i.e. the most complex model on which other models are nested) and assessing the fit of this model in two ways: i) by means of a Pearson chi-square test (MacKenzie and Bailey, 2004), using a parametric bootstrap procedure (5000 re-samplings), ii) by inspecting residuals (Knape et al., 2018). In order to avoid overfitting and creating too many models, deriving from the combinations of covariates for each of the four parameters of the Dail Madsen (2001) model, which can lead to uninformative and biologically unsound models, we preferred to build fewer models in a stepwise approach, considering one parameter at a time, and building biologically informative models. We proceeded modelling the detection probability, considering it to be constant, time-dependent, or to be affected by climatic variables. Then we modelled the initial abundance as a function of site-specific covariates (elev, city and site) or constant over sites. Finally, we considered the survival to be influenced by the same site covariates as abundance, or constant over sites. For each model we considered recruitment as constant. We ranked all models with Akaike's Informative Criterion corrected for small samples (AICc). We conducted model selection and considered only models with ΔAICc > 2 (Burnham and Anderson, 2002). Modelling was conducted in the R environment with package Unmarked (Fiske and Chandler, 2011) and AICcmodavg (Mazerolle, 2017).

3. Results

In 2017 we counted a total of 131 male frog calls during three surveys (44; 45; 42; respectively), while in 2018 we counted 129 male frogs during four surveys (33; 37; 32; 27; respectively, Supplementary Material Table A1). The global model had a good fit (goodness-of-fit, p = 0.34; c-hat overdispersion = 1.12, and visual inspection of residuals). Model building procedure produced a total of 15 models (Table 2). The most supported model included elevation as a covariate on the initial abundance, highlighting a negative effect of elevation (β-elev = -0.331; 95% CI = -0.59 to -0.08; Figure 1). The estimated mean frog abundance per site was 3.4 (95% CI = 2.5 – 4.6). Individual detection probability for this model was constant, and estimated as p = 0.53 (95% CI = 0.42 – 0.63). Survival probability between years was considered constant among sites and resulted ω = 0.71 (95% CI = 0.50 – 0.86). Finally, the recruitment rate was constant across sites γ =

0.11 (95% CI = 0.00 – 12.10). From this best model we also obtained, as a derived parameter from the posterior distribution of the latent abundance, the total abundance of surveyed sites, which resulted of 89 frogs in 2017 (95% CI = 81 – 147) and 64 frogs in 2018 (95% CI = 60 – 109).

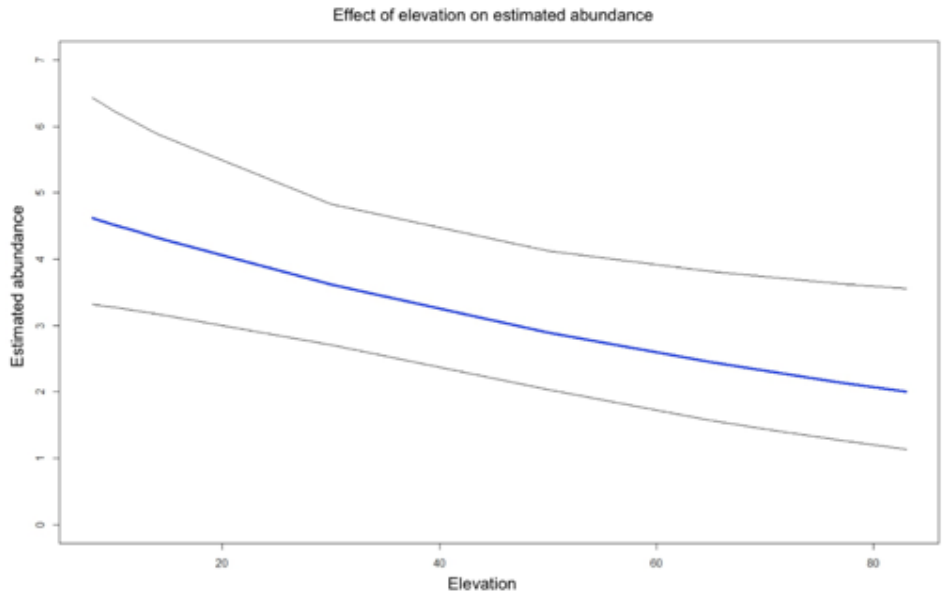
Model	Parameters	AICc	ΔAICc	AICc*WT*
λ(elev) *p*(.) ω(.) γ(.)	5	451.35	0.00	0.40
λ(elev) *p*(.) ω(elev) γ(.)	6	453.45	2.1	0.14
λ(city) *p*(.) ω(.) γ(.)	6	453.98	2.63	0.11
λ(elev) *p*(.) ω(city) γ(.)	6	454.95	3.61	0.07
λ(.) *p*(.) ω(.) γ(.)	4	454.96	3.61	0.07
λ(site) *p*(.) ω(.) γ(.)	5	455.05	3.70	0.06
λ(elev) *p*(.) ω(site) γ(.)	7	456.15	4.80	0.04
λ(.) *p*(temp) ω(.) γ(.)	5	456.81	5.46	0.03
λ(.) *p*(.) ω(site) γ(.)	5	456.93	5.59	0.02
λ(.) *p*(rh) ω(.) γ(.)	5	457.14	5.80	0.02
λ(.) *p*(wind) ω(.) γ(.)	5	475.54	6.20	0.02
λ(.) *p*(rain) ω(.) γ(.)	5	458.03	6.69	0.01
λ(.) *p*(.) ω(elev) γ(.)	5	459.15	7.80	0.01
λ(.) *p*(.) ω(city) γ(.)	6	459.35	8.01	0.01
λ(.) *p*(t) ω(.) γ(.)	11	483.07	31.73	0.00

Table 1 Candidate N-mixture open population models (Dail and Madsen, 2011) used to estimate Hyla meridionalis abundance, ranked by AICc. γ = recruitment rate; λ= initial site abundance; p = individual detection probability; ω = survival. In model list t stands for time dependence. For covariate abbreviations see Table 1.

4. Discussion

Our study showed that N-mixture modelling applied to individual frog calls can be successfully used to estimate male population size together with demographic parameters and ecological understandings. In the CTNP, where *H. meridionalis* is a species of high conservation concern, the male tree-frog population size showed no significant change between years, and site abundance was negatively related with altitude (Salvidio, 2007; Sillero, 2010). Moreover, the usefulness of *N*-mixture approach may be appreciated by comparing population estimates corrected by detectability to raw counts that, in the present case, underestimated the total

Figure 1 Effect of elevation on site specific abundance of Hyla meridionalis in the Cinque Terre National Park, with 95% confidence intervals, obtained by N-mixture open population modelling (Dail and Madsen, 2011)



number of males by about 45%, in both years. Another important application of N-mixture population open models (Dail and Madsen, 2011) relies on the possibility of estimating temporal variations in inter-annual population size, this information being of interest in conservation and management programmes concerning protected species characterised by low or variable detection probabilities (Ficetola et al. 2018). Conversely, the major limits of our study were that the occurrence of calling males does not always assure for the presence of a breeding site, while no data on population structure (i.e. population sex ratio and proportion of juveniles) can be provided (Dorcas et al., 2009). In any case, *N*-mixture models are cost-effective alternatives to mark-recapture and removal sampling methods (Kéry and Royle, 2015; Kéry, 2018), and they have been used to estimate population size and temporal trends of many species in very different ecological contexts (e.g. Priol et al., 2014; Romano et al., 2017; Kéry, 2018; Costa et al., 2019). However, to our knowledge there are few applications of *N*-mixture modelling to anuran call counts, because of the difficulties in correctly counting calling males in large frog choruses when dozens of calls are synchronous (Weir and Mossman, 2005). Nevertheless, when few individual males are calling at each site the application of the N-mixture modelling seems useful and can be preferred to other methods that estimate population abundance because there is no need to mark and recapture the focal individuals (Royle, 2004a; Royle and Link, 2005).

Aknowledgments

The constructive comments of two anonymous reviewers on a previous draft of the manuscript are appreciated. This research was funded by the Cinque Terre National Park within the programme "Azione di Sistema - Monitoraggio delle specie di habitat umidi-acquatici".

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Chapter 5

Reliability of multinomial N-mixture models for estimating abundance of small terrestrial vertebrates

Part of this chapter is under review as: Costa, A., Romano, A., Salvidio, S. Reliability of multinomial N-mixture models for estimating abundance of small terrestrial vertebrates. *Biodiversity and Conservation.*

Abstract

Information on population size are important to correctly plan monitoring, conservation and management of animal populations. In general, capture-mark-recapture (CMR) is considered the most robust technique to estimate population abundance, but it is costly in terms of time and effort. Recently, binomial N-mixture models, based on counts of unmarked individuals, have been widely employed to estimate abundance. These models have limits and their reliability has been criticized. In some cases, multinomial N-Mixture models, based on multiple observer protocols, that are hierarchical extensions of simple CMR, are applied in estimating abundance of animals with large body size, conspicuous behavior and high detection probabilities. In this study, we applied and evaluated the reliability of a multinomial N-Mixture modelling approach to a small and cryptic terrestrial salamander, found in different habitats where populations possess different level of detectability. Estimates obtained with multinomial N-Mixture models were compared to estimates obtained with classical methods, such as removal sampling, and their reliability has also been evaluated by simulations scenarios. Our results show that multinomial N-Mixture models, applied within a multiple observer framework, give reliable and robust estimates of population size even when detection and density are low. Therefore, multinomial N-Mixture models appear cost-effective when planning large-scale monitoring programs of small terrestrial animals such as amphibians and reptiles.

1. Introduction

Accurate information on population size are of paramount importance in the conservation, monitoring and management of animal populations (Seber, 1982; Yoccoz et al., 2001; Williams et al., 2002). Among the variety of methods proposed to estimate population abundance, capture-mark-recapture (CMR) protocols are considered the most robust techniques (Lincoln, 1930; Seber, 1982; White et al., 1982; Williams et al., 2002; Barker et al., 2017). CMR methods are based on the proportion of individually marked animals that are recaptured or re-sighted over successive sampling occasions. In CMR studies, individual identification of the study animals through natural or artificial marks is needed for the reason that it is highly unlikely to capture or sight all the individuals of a wild population. Indeed, an unknown fraction of the population of interest will not be available to the observer during sampling (Nichols and Conroy, 1996; Borchers et al., 2002; Schmidt, 2003). Therefore, CMR models are based on the encounter history of each captured individual, allowing the estimation of the entire population size through the calculation of capture probabilities, or detectability (Seber, 1982; White et al., 1982; Schmidt, 2003). However, in some cases, animals may be not marked physically, but just sighted and recorded by two or more independent observers, with a protocol that allows to partition the sightings into those scored by each observer and those scored in common by all observers (i.e., Cook and Jacobson, 1979; Southwell, 1996). These adaptations of the CMR framework, are known as "multiple observers" methods (Table 16 in Southwell, 1996). Methods, using two or more dependent or independent observers, have been applied specifically to animals readily observable from long distances, such as waterfowl (e.g., Koneff et al., 2008; Vrtiska and Powell, 2011), large terrestrial or marine mammals (e.g., Cook and Jacobs, 1979; Langtimm et al., 2011; Broker et al., 2019) or to animal traces and signs, such as amphibian egg masses that possess high detection probability (e.g., Grant et al., 2005). Recently, CMR models have been applied within a hierarchical, meta-population design, within the multinomial N-mixture modelling framework, which is a generalization of the binomial N-mixture models proposed by Royle (2004b). Binomial N-mixture models estimate population abundance from spatially and temporally repeated counts of unmarked individuals, considering a binomial detection process for the observation as a part of the model (Royle, 2004b; Kéry and Royle, 2016), meaning that only two outcomes are possible: an individual can be detected, or not. Multinomial N-mixture models, instead,

using different sampling frameworks, allow for multinomial outcomes of the detection process: e.g. under a double observer protocol, an individual could be either detected by observer #1, by observer #2 or by both observers (#1+#2). Recently, the reliability of binomial N-mixture models has been criticized, mainly because parameter identification issues in case of assumption violations and unmodeled heterogeneity on the detection or abundance processes (Barker et al., 2017; Link et al., 2018). While some skepticism is still present about the reliability of binomial N-mixture models, despite many studies successfully compared this method with reference techniques for abundance estimation (Ariefiandy et al., 2014; Priol et al., 2014; Ficetola et al., 2018; Costa et al., 2019), there should be no doubt about parameter identifiability in multinomial N-mixture models (Kéry, 2018). These models rely on the unique encounter histories of the individuals detected over multiple sampled sites (Royle, 2004a; Royle and Dorazio, 2006; Chandler et al., 2011; Kéry and Royle, 2016) and, therefore, multinomial N-mixture models can be considered hierarchical extensions of simple CMR models (Kéry and Royle, 2016; Kéry, 2018). To date, multinomial N-mixture models have been applied within different sampling protocols, such as removal sampling (i.e., bird point counts, Kéry, 2018), double observer sightings from aircrafts and distance sampling applied to large mammals (i.e., manatees, Langtimm et al., 2011; narvhals, Broker et al., 2019). However, in all cases the focal animals were detectable from a distance by sighting or hearing, and possessed relatively high detection probabilities, due to their conspicuous behavior and or large body size. To our knowledge, double-observer multinomial N-mixture models have not been yet applied in the estimation of population abundance of small terrestrial animals, such as amphibians and reptiles. In fact, these animals are mimetic and cryptic and, therefore, have to be captured or observed from a close distance to be correctly identified. For these reasons, amphibians and reptiles are often characterized by low detection probabilities (e.g., McDiarmid et al., 2012; Griffiths et al., 2015). In this study, we applied and evaluated the reliability of a multinomial N-Mixture modelling approach to a small and cryptic terrestrial salamander, found both in underground environments and on the forest floor in SW Europe (Costa et al., 2016; Salvidio et al., 2017). Salamander population abundance was estimated in simplified habitats (i.e., artificial caves) and also in highly complex habitats (i.e., on the forest floor of mixed broadleaf woodlands). In these two habitat types, salamanders' detection probabilities are known to be different, higher in caves and lower in

woodlands (Salvidio, 2001, 2007; Lindström et al., 2010; Costa et al., 2016; Salvidio et al., 2016). In both these habitats we compared estimates from double observer multinomial N-mixture models with those obtained with other reference methods. The application of multinomial N-mixture models to the monitoring and conservation of amphibian populations is then discussed on the basis of the observed results.

2. Materials and methods

2.1 Study rationale and framework

Aim of this study is to evaluate the reliability of multinomial N-mixture models applied with a double observer sampling protocol for abundance estimation of small and cryptic vertebrates, possessing low densities and low detection. In order to select a representative study species, we focused on a European plethodontid salamander, *Speleomantes strinatii* (Aellen, 1958), inhabiting different environments, and thus experiencing different levels of density and detection probability. For this purpose, we selected two habitats: a subterranean environment (i.e. artificial caves), where detection probability of the focal species is usually high (mean capture probabilities $\geq$ 0.50, Salvidio, 2001; Lindstrom et al., 2010), and a complex woodland environment, where detection probabilities are markedly lower (mean capture probabilities < 0.40; Salvidio, 2007; Costa et al., 2016). Abundance estimates obtained by multinomial N-mixture models in the subterranean environment were compared with those obtained by removal sampling (White et al., 1982). In the woodland habitat, where reference techniques for abundance estimation were not available, we estimated abundances with double observer multinomial N-mixture model and compared estimates with those obtained from a similar model, but performed with three observers. We also performed simulations under several scenarios in order to: i) assess the reliability of a triple observer survey to serve as a reference metric for a double observer method, by comparing the bias in abundance estimation for both methods, under the same conditions, ii) to assess the bias in abundance estimation for the double observer method, under the conditions commonly encountered when dealing with small terrestrial vertebrates, also taking into account unmodelled heterogeneity in abundance across sampling locations (Kéry and Royle, 2016), iii) to evaluate the reliability of a parametric bootstrapping based goodness-of-fit test (GOF), commonly used as a diagnostic tool for model adequacy in N-mixture and Occupancy models to our multinomial N-Mixture simulations (MacKenzie and Bailey, 2004; Kéry and Royle, 2016; Duarte et al., 2018).

Subterranean environment	**Cave 1**		
	Abundance *N-hat* (CI)	Detection *p* (CI)	
	95 (74 – 116)	Observer A 0.68 (0.57 – 0.78)	Oberver B 0.83 (0.72 – 0.90)
	Cave 2		
	Abundance *N-hat* (CI)	Detection *p* (CI)	
	140 (117 – 166)	Observer A 0.81 (0.73 – 0.87)	Observer B 0.87 (0.79 – 0.92)
Woodland environment	**Three Observers**		
	Abundance λ (CI)	Detection *p* (CI)	
	1.44 (0.92 – 2.23)	Observers A + B + C 0.37 (0.24 – 0.52)	
	Observers A B		
	Abundance λ (CI)	Detection *p* (CI)	
	1.33 (0.71 – 2.53)	Observers A + B 0.38 (0.18 – 0.63)	
	Observers A C		
	Abundance λ (CI)	Detection *p* (CI)	
	1.68 (0.88 – 3.21)	Observers A + C 0.35 (0.16 + 0.58)	
	Observers B C		
	Abundance λ (CI)	Detection *p* (CI)	
	1.04 (0.59 – 1.82)	Observers B + C 0.48 (0.25 – 0.70)	

Table 1 Abundance and detection probability estimates, and 95% Confidence Intervals, for two subterranean sites and one woodland site, obtained by means of multinomial N-mixture models.

2.2 Study species

The European plethodontid *Speleomantes strinatii*, is a fully terrestrial salamander found in S France and in NW Italy (Lanza, 2007). This medium-sized species is about 115 mm in total length and shows a cryptic dorsal ground coloration with irregular ochre spots (Lanza, 2007; Salvidio et al., 2017). In the study region, the species is found in humid forest habitats along small streams, but also in humid underground habitats such as natural and artificial caves (Salvidio et al., 2017). Here over the years, population abundance has been estimated both by temporary removal sampling and by binomial N-Mixture modelling (Salvidio 2001, 2007; Costa et al., 2016; Salvidio et al., 2016).

2.3 Study sites

The two experimental caves are subterranean horizontal tunnels located in NW Italy and extending 39 and 16 m underground, respectively. Both caves were divided in sections with a length of 1 m and extending on both side walls and on the ceiling. The first cave (Cave 1) is the experimental Biospeleological Station of S. Bartolomeo (Municipality of Savignone, Province of Genova, Liguria) where a permanent grid, with 1-m mesh, is set up to 21 m from the entrance (Salvidio et al., 1994). The second cave (Cave 2) is located at about 10 km from the first one, in the municipality of Isola del Cantone (Province of Genova, Liguria). The woodland site is located in the Municipality of Carrega Ligure (Province of Alessandria, Piemonte) at 900 m a.s.l and is about 16 km from the nearest study cave. This woodland is located on a small valley, crossed by a first order Apennine stream, and characterized by a supra-mediterranean mixed deciduous forest (Blondel and Aronson, 1999). In this habitat, 25 square plots with a surface of 9 m^2 were randomly selected and then marked at their corners with red flags, while during sampling, two ropes crossing at the center of the plot, delimited four quadrants to facilitate the exact recording of salamanders' positions, within the plot.

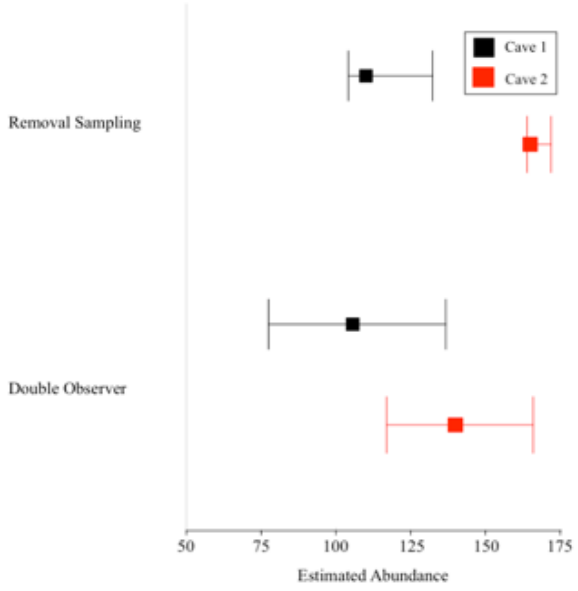

Figure 1 Forest plot comparing total population estimates (N-hat), along with 95% Confidence Intervals, of Removal Sampling and multinomial N-mixture models with double observer protocol.

2.4 Salamanders' sampling

In July 2019, two observers (A and B) independently searched with the aid of head lamps all the 1-m sections in which the two caves were divided, and recorded the number and the position of each observed salamander. Then, after searching in each section, A and B immediately reconciled their observations, and compiled a complete data sheet. These sheets reported all possible outcomes of the detection process, that consisted of three possible individual encounter histories: an individual could either be detected by observer A only (1,0), by observer B only (0,1), or by both observers (1,1). Starting from the day after the application of this protocol, the salamanders were removed from the cave walls during three temporary removal samplings performed every other day (i.e., within 72 hours), as described by Lindström et al. (2010). Animals were kept in terraria inside the caves and returned to their capture sites at the end of the third removal session.

In the woodland, the survey was conducted in October 2019, during a humid and rain period, to maximize salamander detection. After locating the marked plots, three observers (A, B and C) independently checked the 9 m^2 plots for a fixed time of 2 minutes each, and registered the number and the exact position of each observed salamander on an ink-board representing the four quadrants of the plot. At the end of each plot search, observers' data sheets were reconciled and recorded on a complete sheet with seven possible individual encounter histories (all possible outcomes of a triple observer protocol).

2.5 Data analyses

Cave removal data were analyzed using model M_{bh} implemented in CAPTURE software (White et al., 1982), obtaining estimates of cave population abundances (*N-hat*), 95% confidence intervals (CI) and capture probabilities (p). Multinomial N-mixture models, include a parameter for the latent variable, i.e. mean abundance at each sampling site (λ), and a parameter for the detection process, i.e. detection probability (p) (Royle and Dorazio, 2006; Chandler et

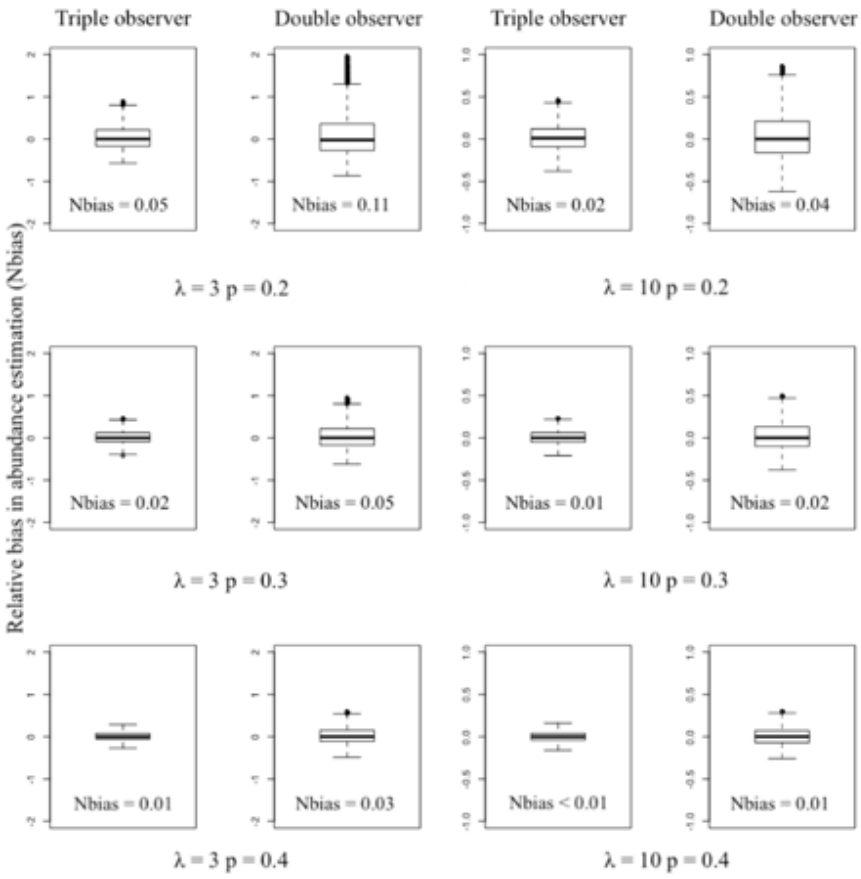

Figure 2 Boxplots comparing the relative bias in abundance estimation (Nbias) between a triple observer protocol and a double observer protocol under the same scenario, within a simulation framework. λ = mean site abundance; p = detection probability

al., 2011). In subterranean environments, data from the double observer protocol were analyzed with a Poisson error distribution for λ and, at the same time, detection probability was modelled either as constant or as observer-dependent. Selection between competing models was carried out by means of Akaike Information Criterion corrected for small sample size (AICc), and considering that models with ΔAICc > 2 show substantial differences (Burnham and Anderson, 2002). When models differed by less of 2 AICc points, model averaged estimates were calculated (Burnham and Anderson, 2002). Total population estimates (*N-hat*) for each cave, along with 95% CI, were obtained by posterior distribution of the latent abundance. In our analysis, the first step consisted in evaluating if a triple observer sampling could lead to better estimates than a double observer protocol. A multiple observer protocol with T observers potentially yields 2^T-1 individual encounter histories (Royle and Dorazio, 2006). This means that double and triple observer protocols will produce three and seven possible observable individual encounter histories, respectively. For this reason, conducting a triple observer sampling rather than a double observer one in the same

conditions, should give a clear advantage, exactly as occurs in CMR studies, where three capture sessions give more robust estimates in comparison to two capture sessions (Williams et al., 2002). In the light of this, we performed a simulation analysis to test the effectiveness of these two protocols to estimate abundance under different scenarios. We generated six different scenarios, with all possible combinations of abundance (λ = *3, 10*) and detection probability (p = 0.2, 0.3, 0.4) values, and considering a study framework with 25 sampling plots. These values of abundance and detection were realistic, because were obtained by previous studies on the same species and in similar subterranean and woodland habitats (Salvidio, 2001; Costa et al., 2016). For each scenario, we built 5000 data-sets for both double and triple observer sampling. We then compared the estimated abundance (n) of each simulation with the real abundance of the scenario (N) and calculated the relative bias as $Nbias = (n - N) / N$. Then, we compared bias in estimates between the two sampling protocols, under the same scenario. In addition, for each simulated data-set of the double observer protocol, we also calculated the coefficient of variation (*CV*) of the real abundance, across sampling locations, and employed it as a measure of heterogeneity (*Nheterogeneity*). We then performed a correlation analysis between *Nbias* and *Nheterogeneity*, in order to assess if observed deviations from real abundance were related to unmodeled heterogeneity in the latent variable. Like any other estimator, also N-mixture models require a set of assumptions to be verified and met. The most adopted method to assess assumption violation in N-mixture and Occupancy models is to employ parametric bootstrapping procedure as a goodness-of-fit test (MacKenzie and Bailey, 2004; Kéry and Royle, 2016). Thus, we performed a GOF test on a subset of our simulation scenarios to evaluate the reliability of parametric bootstrap GOF test in identifying bias in parameter estimation. We simulated again our six scenarios for double observer protocol, but this time we also performed a Pearson chi-square test using a parametric bootstrap procedure with 1000 resampling, for each fitted model (MacKenzie and Bailey, 2004). We considered models to result in a good fit when the p-value of the Pearson chi-square was ≥ 0.1, indicating that frequencies of observed and simulated encounter histories do not differ significantly. Moreover, for each fitted model, we also obtained a measure of overdispersion (*c-hat*) which considers that values will converge or diverge from 1.0, when models are showing good fit or violation of assumptions, respectively. Then, we compared the relative bias in abundance estimation of each model with its

corresponding GOF test, highlighting whether GOF test is a good predictor of model bias. Moreover, we divided the data from GOF test simulations in two groups: i) significant, with a *p*-value of the Pearson chi-square test < 0.1 ii) and non-significant, having a *p*-value ≥ 0.1. We then tested if *Nbias* differed between groups, by means of a Mann-Whitney test. For these analyses, we used 2000 simulations for each scenario.

Real data on woodland salamanders, collected with the triple observer protocol, were split in three double observer data-sets (all possible combinations of observers A, B and C). Double and triple observer data-sets were analyzed by mean of multinomial N-Mixture modelling, following the same approach used for cave populations. We obtained abundance estimates from the triple observer protocol and all the possible double observer combinations and we compared them. Multinomial N-mixture models were run on package "*unmarked*" (Fiske and Chandler, 2011), while model selection, model averaging and GOF testing were carried out in package "*AICcmodavg*" (Mazerolle, 2017), both in R environment. In order to fit multinomial N-mixture models with triple observer sampling protocol we employed a custom function for calculating the multinomial cell probability (available in Supplementary Material Appendix 1, Function 1).

3. Results

During the three removal occasions, we captured 59, 21 and 12 individuals in Cave 1 and 126, 26 and 10 individuals in Cave 2. In these same environments, during the application of the double observer protocol, we counted a total of 90 and 137 salamanders, in Cave 1 and 2, respectively. In both caves the multinomial N-Mixture model selected according to its AICc value was the one with observer-dependent detection probability (Supplementary Material Appendix 1, Table 1). In the two caves, abundance estimates from removal sampling were *N-hat* = 98 (95% CI = 94 – 113) and *N-hat* = 165 (95% CI = 164 – 172), while those estimated from the double observer protocol were *N-hat* = 95 (95% CI = 76 – 116) and *N-hat* = 140 (95% CI = 117 – 166) for Cave 1 and 2, respectively. In both cases, the double observer method underestimated salamander abundance in comparison to removal sampling, but at the same time 95% CI largely overlapped (Figure 1). Results from the simulation study showed how the overall bias in abundance estimation was low in all the considered scenarios, the worst scenario experiencing a mean relative bias = 0.11. Furthermore, the triple observer protocol

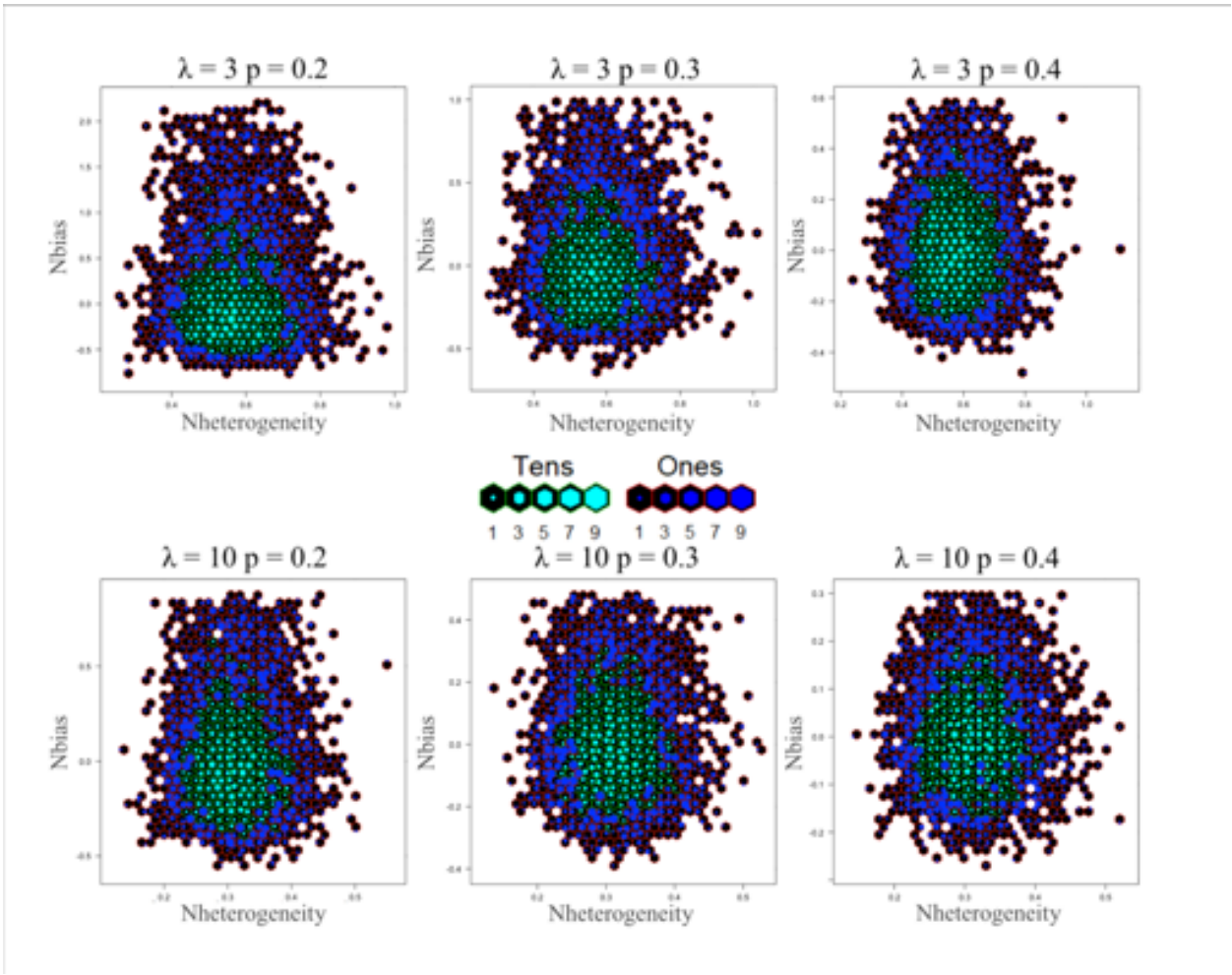

Figure 3 Scatterplots representing the relationship between relative bias in abundance estimation (Nbias) and the coefficient of variation of abundance across sampling locations (Nheterogeneity), under different scenarios. Hexagonal bins represent the number of points falling within the hexagon surface. λ = mean site abundance; p = detection probability.

had a very low bias in abundance estimation, that was almost halved when compared to double observer (Figure 2). *Nheterogeneity* across sampling sites, in our simulation framework, ranged between 0.14 and 1.07 (*mean* = 0.44; *SD* = 0.15). No relationship was observed between *Nbias* and *Nheterogeneity* (Figure 3) and Pearson's product-moment correlation was not significant for any scenario ($p > 0.05$; Supplementary Material Appendix 1, Table 2). Results from simulations also highlight the low performance of the parametric bootstrap GOF test as a predictor of model bias, being both *p*-value and *c-hat* unrelated with bias (Supplementary Material Appendix 1, Figure 1). Moreover, the mean *Nbias* did not differ between significant and non-significant models (Mann-Whitney test; p = 0.89). On the forest floor, we encountered a total of 27 individuals in 19 of the 25 surveyed plots. Estimates of woodland salamander models are reported in Table 1 (model selection is reported in

Supplementary Materials Appendix 2). Abundance estimates of woodland salamanders, obtained from all pairs of observers, were in good agreement with those obtained from the triple observer protocol (Figure 4). Finally, the heterogeneity in estimated abundance, across sites, for this data-set ($CV = 0.63$) was similar to the mean *Nheterogeneity* observed in the simulation framework.

4. Discussion

Multiple observer protocol is usually applied to large and conspicuous animals, or to animal signs, all characterized by high or very high detection probabilities (e.g. $p > 0.80$). By contrast, we applied this technique to a small, secretive vertebrate in different environments and thus under different conditions of density and detection probability. In subterranean habitats, given the low complexity of the environment, cave salamanders typically exhibit a high detection probability, as reported by Salvidio (2001; mean $p = 0.63$) and Lindstrom et al. (2011; $0.60 < p < 0.72$). Conversely, in more complex environments, such as forest floors, these salamanders are known to exhibit relatively low detection probability. For instance, Costa et al. (2016) report a detection probability $p = 0.34$, and Salvidio (2001) reports a capture probability $p = 0.33$, for two woodland populations of *Speleomantes strinatii* living in woodland habitats similar to the one sampled in our study. These levels of detectability, reported in literature, are in good agreement with those experienced in this study for both environments. By comparing abundance estimates of multinomial N-mixture models in the caves, with those obtained from removal sampling, we can observe that the two methods are in good agreement. Nevertheless, in both caves, multinomial N-mixture models slightly underestimated salamander abundance. This outcome could be caused by two sources: i) if intrinsic heterogeneity (i.e. structured variation in the detection process related to density dependence or individual behavior) is present, then underestimation can be common and severe (Veech et al., 2016), or ii) double observer protocol is carried out on a single session and can be considered an instantaneous estimate of the population abundance, while removal sampling may span over several days and, therefore, includes parts of the population unavailable during previous removals. Considering the application of the multiple observer protocol to woodland sites, our simulation study showed that a protocol with three independent observers had a *Nbias* markedly lower than a double observer one, under different but realistic conditions of detectability and abundance. In our case study, estimates from each pair of observers were in good agreement, and similar to the

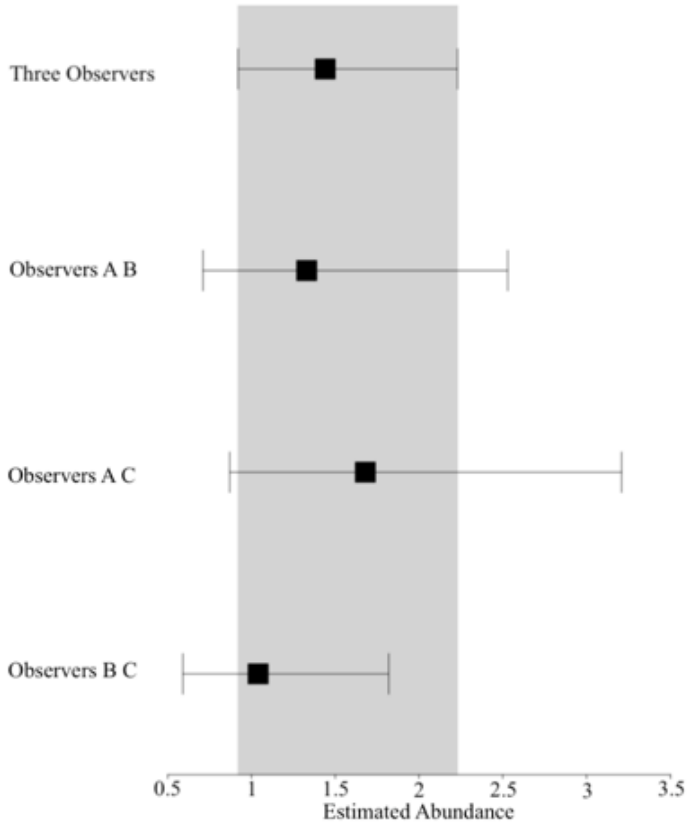

Figure 4 Forest plot comparing estimates of site abundance (λ) and 95% Confidence Intervals, obtained from a multinomial N-mixture model with a double observer protocol, using all pairs of observer (A, B, C), to those from the same model with a triple observer protocol. Shaded box represents 95% CI of the triple observer sampling protocol.

triple observer method. Under these conditions of abundance and detection, considering the additional effort, sampling with three observers was not cost-effective, in comparison with the double observer protocol. Conversely, in condition of lower detection probability, our simulation study suggests that a triple observer approach may significantly reduce *Nbias* and, therefore, give some advantage in estimating population abundance. In recent years, binomial N-mixture models have been criticized, one of the major issues being due to lack of robustness to unmodelled heterogeneity in the data (e.g., Barker et al., 2017; Link et al., 2018). Indeed, many authors showed that intrinsic heterogeneity in the detection process (i.e. the variation in detection probability among individuals), and the heterogeneity in abundance, (i.e. variation in abundance among surveys), can lead to a large bias in parameter estimation (Veech et al., 2016; Link et al., 2018; Duarte et a., 2018). This kind of heterogeneity is comparable with some sort of assumption violation, such as population closure or constant detection among individuals (Royle, 2004b), and therefore cannot be

addressed by the use of covariates. Another kind of heterogeneity is given by the variability of abundance between sampling sites or plots, that if not properly modelled, could lead to biased abundance estimations (Kéry and Royle, 2016). By contrast, little is known about the effect of heterogeneity in multinomial N-mixture model. In the present study, from our simulation framework, we showed how the relative bias in abundance estimation is unrelated to the unmodelled heterogeneity of abundance between sampling locations (*Nheterogeneity*). Although we didn't specifically generate simulated data-sets taking into account *Nheterogeneity* in our study, our simulations spanned along a wide range of heterogeneity in abundance, similar to the one really observed for the study species in the field conditions, and higher than the one used by some authors, specifically testing the effect of heterogeneity on binomial N-mixture models (Duarte et al., 2018). Thus, we can conclude that, at least for the range of conditions considered within our simulation and real-life study, and unlike binomial N-mixture models, unmodelled heterogeneity in abundance is not an issue for multinomial N-mixture models. A diagnostic tool for assessing model adequacy, and therefore preventing the use of unreliable estimates, should be of paramount importance in ecological modelling (Burnham and Anderson, 2002). In hierarchical models used in the study of population ecology, whether they are Occupancy or N-mixture models, the most adopted tool for diagnosing model fit is a parametric bootstrap-based GOF test (MacKenzie and Bailey, 2004). Duarte et al. (2018) already casted several doubts about the reliability of this GOF test for evaluating binomial N-mixture models, reporting how the test is unreliable in both identifying bias in parameter estimation and assumption violations. Again, with our simulation study, we also evaluated the effectiveness of this GOF test and showed how, also for the multinomial N-mixture models, it is quite unreliable in identifying model inadequacy and, therefore, high level of bias in abundance estimation. As already stated by other authors (Duarte et al., 2018; Knape et al., 2018) in relation to binomial N-mixture models, we suggest to focus on the development of alternative diagnostic tools for assessing model fit, also for multinomial N-mixture models. Binomial N-mixture models, being based on count data, and not requiring additional information, have been widely adopted for their cost-effectiveness and ease of application (Ariefiandy et al., 2014; Romano et al., 2017). However, the need for temporal replication, or additional effort for space-for-time substitutions (Royle, 2004b; Kéry and Royle, 2016), is costly in terms of time and human resources. Multinomial N-mixture models in general, and double observer N-mixture models in

particular, do not require temporal replication and, for this reason, are even more cost-effective, being robust at the same time, and it seems especially suitable in situations where temporal replicates are particularly costly, such as in areas difficult to access. Therefore, we suggest that they should be taken seriously into consideration when planning large-scale animal population monitoring for management and conservation, also for small, inconspicuous, vertebrate species characterized by a low detection probability.

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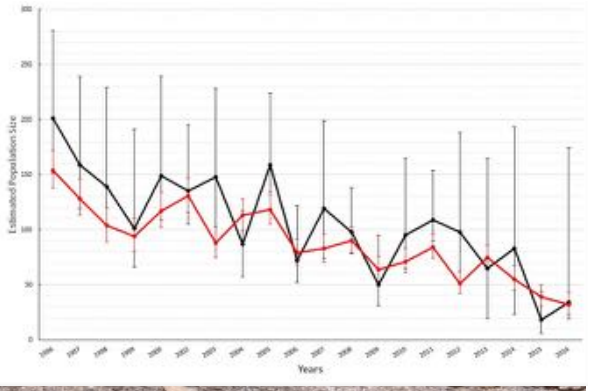

PART THREE - Time-for-space substitution in N-mixture model for long term monitoring

Chapter 6

Time-for-space substitution in N-mixture modeling and population monitoring: comparison with twenty years of capture-mark-recapture data

Part of this chapter is published as: Costa, A., Oneto, F., Salvidio, S. (2019) Time-for-space substitution in N-mixture modeling and population monitoring. *The Journal of Wildlife Management 83, 737 - 741.*

Abstract

Population size is a fundamental state variable in ecology, and the analysis of temporal variation in abundance (i.e., detection of trends) is a prime objective in wildlife monitoring. However, population abundance cannot be directly observed because part of the population remains undetected and methods that account for imperfect detection are often not used. Capture-Mark-Recapture approaches give reliable estimates of abundance, but are time- and effort-consuming. In the last decade, the application of hierarchical, or *N*-mixture, models that use repeated counts of unmarked animals seem to give great advantages in the estimation of population size. Hierarchical models require repeated surveys at multiple sites, but sometimes only data obtained for a single site in successive years are available. We applied the time-for-space substitution implemented within the *N*-mixture modeling framework, to estimate population size and evaluate the dynamics of the endangered European leaf-toed gecko (*Euleptes europaea*) surveyed >20 years. We compared these results with capture-mark-recapture estimates obtained from the same population and over the same time period. Estimates and trends were comparable and both methods indicated similar population declines, moreover N-mixture modeling indicated temperature affected detection. Therefore, the application of the time-for-space substitution in hierarchical modeling seems valuable and may be useful in species monitoring and conservation.

1. Introduction

Population size is one of the fundamental state variables in ecology and the analysis of its temporal variation in abundance (i.e., detection of trends) is a major objective in wildlife monitoring and species conservation (Yoccoz et al. 2001, Williams et al. 2002). However, population abundance usually cannot be directly observed because part of the population of interest may remain undetected (Schmidt 2002, Williams et al. 2002). Therefore, methods that account for imperfect detection, such as capture-mark-recapture (CMR) or removal methods, should be employed to obtain robust population estimates (Williams et al. 2002). These approaches give reliable estimates of abundance and other demographic parameters but are time- and effort-consuming. In the last decade, the application of *N*-mixture models (Royle 2004), which use repeated count data without the need of individual capture and identification, seem to give great advantages for estimating population size with reduced field effort. *N*-mixture models received a great interest in the last few years and their reliability has been evaluated by simulation studies, casting doubts on the usefulness of these models because of parameter identifiability problems; in particular in presence of assumptions violation and unmodeled heterogeneity in the abundance or the detection parameter (Barker et al. 2017, Link et al. 2018). However, Kéry (2018) showed how binomial N-mixture model estimates are in agreement with those obtained with a hierarchical variant of a capture-recapture model. Finally, several studies compared N-mixture models with other techniques for abundance estimation such as CMR or removal methods, obtaining comparable results (Priol et al. 2014, Ficetola et al. 2018). *N*-mixture models are typically used for repeated surveys at multiple sites (Kéry and Royle 2016), but sometimes only monitoring data for single sites or populations obtained in successive years are available. Time-for-space substitution in *N*-mixture models, where multiple counts are conducted each year at the same site, consists of substituting space replicates (i.e., sites) by time replicates (i.e., years), and within-year repeated counts (i.e., surveys) are employed as temporal replications (i.e., the population is considered demographically closed within each year). This framework has been employed by Yamaura et al. (2011) for a multi-species system with detection–non-detection data of bird species during 9 consecutive years at a single site in Japan. This application is also described and evaluated against simulation scenarios by Kéry and Royle (2016), but further applications with real field data and, in particular, a validation of the method with

another one based on CMR techniques are lacking. We applied the time-for-space substitution approach proposed by Yamaura et al. (2011) within the *N*-mixture modeling framework to estimate the abundance and trend from 20 years of repeated sampling data of a single population of European leaf-toed geckos (*Euleptes europaea*; Gené, 1839) monitored since 1996, in northwest Italy (Salvidio and Oneto 2008). We also compared the population estimates obtained by *N*-mixture modelling with time-for-space substitution with population estimates obtained from the same population and the same 20-year time frame, estimated by the CMR approach in order to evaluate the performance of N-mixture models in this particular context.

2. Materials and methods

2.1 Study area

We monitored European leaf-toed geckos annually since 1996 on an abandoned historical building in the outskirts of the town of Genova, Liguria, northwest Italy, at an elevation of 320 m above sea level and about 4 km from the sea coast. Results of this monitoring have already published and described the study area (Salvidio and Delaugerre 2003, Salvidio and Oneto 2008, Salvidio et al. 2011). The study site is relatively isolated and surrounded by pastured grasslands, interspersed with houses and sparse trees. The climate of this area is submediterranean, with a mean annual rainfall of 1,303 mm and a relatively dry and hot period in July, when <40 mm of mean monthly rainfall are recorded (Genova – meteorological station of Ponte Carrega, Agenzia Regionale per la Protezione dell'Ambiente Ligure [ARPAL] 2013).

2.2 Study species

The European leaf-toed gecko is a diminutive (max. snout-cloaca length = 48 mm; mass < 2 g) nocturnal lizard endemic to the northwest Mediterranean area. It is found on the coastal mainland of northwest Italy and southern France on large (i.e., Sardinia and Corsica) and small offshore islands and on some islets off the coasts of northern Tunisia (Delaugerre et al. 2011, Salvidio et al. 2011). This gecko is a narrow crevices specialist, living on rock cliffs and stony habitats, but it is also able to colonize artificial habitats, such as abandoned buildings and dry-stone walls (Salvidio et al. 2011). The species' altitudinal distribution ranges from sea level to about 1,500 m in Corsica but never goes beyond 900 m on the mainland (Salvidio et al. 2011). The European leaf-toed gecko is a species of conservation concern, has been evaluated as Near Threatened by the International Union for Conservation of Nature (Corti et al. 2009), and is listed in Annex II and IV of the

European Habitats Directive (92/43/EEC), therefore deserving protection in the entire European Union.

2.3 Capture-Mark-Recapture

We sampled the gecko population each year in July from 1996 to 2016, during 3 or 4 non-consecutive nights, with the exception of 2001. We spotted geckos with flashlights, captured them on building walls, sexed and measured them, and temporarily marked them with acrylic paint (Salvidio and Delaugerre 2004, Salvidio and Oneto 2008). At the end of each nocturnal survey, we released all the geckos on the building and did not observe mortality related to capture. The number of operators varied among nights and years, but in all cases captures terminated after 2 completely unsuccessful searches on the building walls. From 1996 to 2009, we batch-marked geckos by painting a single dorsal spot with a different color each night, whereas from 2010 to 2016 we individually marked all animals with a progressive acrylic number painted on their back. In all years captures were executed with permits of the Italian Ministry of Environment. We estimated population abundance by means of Program CAPTURE, a software suited for closed populations (White et al. 1982) that performs a population closure test and a model selection procedure for all available models but only when the complete CMR matrix is available (i.e., full individual capture histories). In the present study, the closure test was non-significant ($P > 0.05$) in all years in which it was applicable, indicating that the population can be considered demographically closed, and in these cases the model selection procedure could be successfully employed. In a previous study in which geckos were batch-marked, Salvidio and Oneto (2008) used the time-dependent estimator, $M_{(t)}$, which allows for variation in capture probabilities among occasions (White et al. 1982). Concerning the data obtained from 2010, the model assuming constant capture probabilities, $M_{(0)}$, was supported in 6 out of 8 years, whereas models $M_{(t)}$ and $M_{(h)}$, the latter allowing individual variation in capture probabilities (White et al. 1982), were selected once each.

2.4 N-mixture model

We conducted *N*-mixture model analyses with the number of geckos captures per night. To minimize stochastic heterogeneity in detection probability (Kéry and Royle 2016) we evaluated several covariates capable of explaining the detection process: temperature (temp), wind speed, relative humidity of the survey night, and the number of surveyors. We built 5 different models, with Poisson error distributions, each

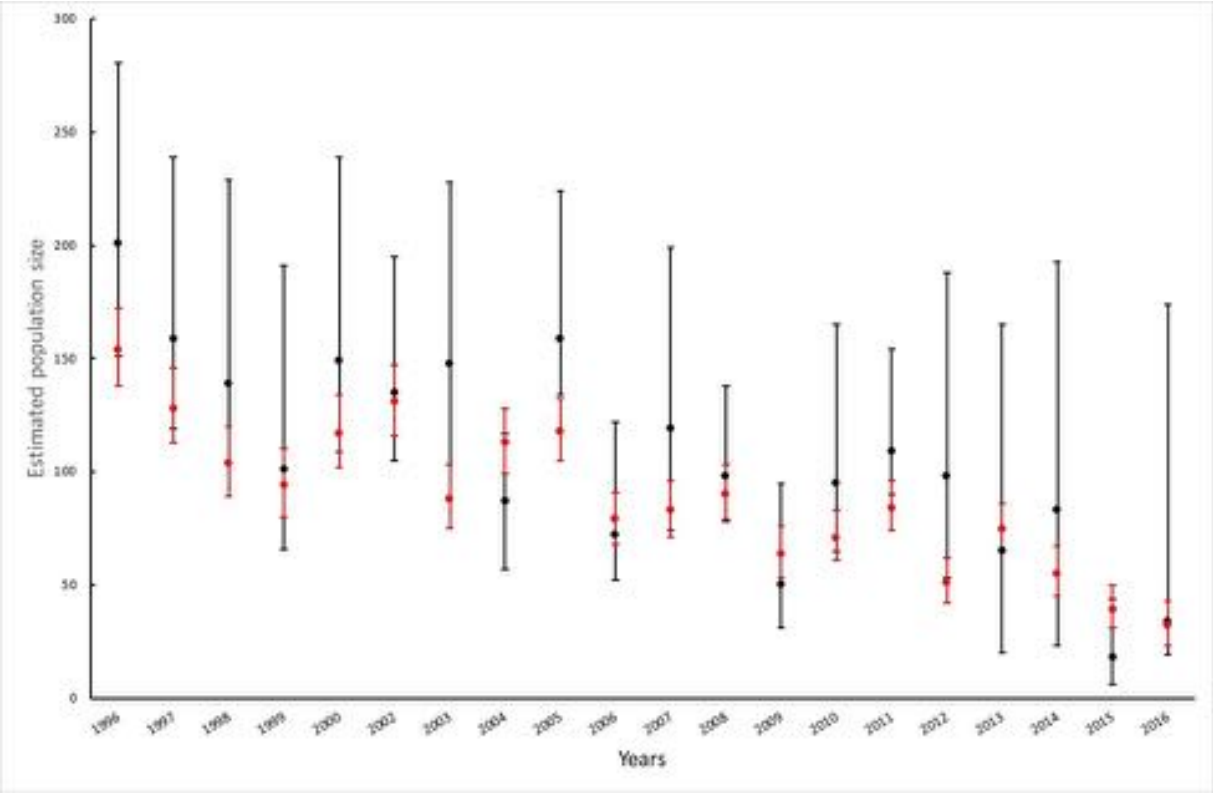

Figure 1 Population trends of the European the leaf-toed gecko Euleptes europaea estimated with capture-mark-recapture (black) and N-mixture models with time-for-space substitution (red). Vertical error bars represent 95% confidence intervals.

including a different covariate for detection probability (plus a model assuming constant detection probability). In each model we added a year numeric variable on the abundance side of the formula to model population trend (Kéry and Royle 2016). We standardized all covariates prior to analysis and assessed collinearity between covariates with Pearson product-momentum correlation (MacNally 2002). We evaluated goodness of fit of the global model (i.e., the model with all the covariates and in which other candidate models are nested) using a Pearson chi-square test (MacKenzie and Bailey 2004), using a parametric bootstrap procedure (5,000 re-sampling). Moreover, we also evaluated model fit by computing a quasi-coefficient of variation (QCV) following Duarte et al. (2018) and inspecting residuals following Knape et al. (2018). We ranked all candidate models with Akaike's Information Criterion corrected for small samples (AIC_c). We conducted model selection and considered models with $\Delta AIC_c > 2$ as having less support than the top-ranked model (Burnham and Anderson 2002). We obtained abundance estimates for each year, with 95% confidence intervals, from the posterior distribution of the latent abundance (function ranef() in package unmarked). We conducted *N*-mixture model analyses in the R environment with package

unmarked (Fiske and Chandler 2011) and package AICcmodavg (Mazerolle 2017).

3. Results

The MacKenzie and Bailey (2004) goodness-of-fit assessment resulted in a good fit (P = 0.26) and estimated a low overdispersion ($\hat{c}$ = 1.08). Likewise, residuals and QVC highlighted a good fit of the model (QCV = 0.11). The most parsimonious *N*-mixture model included night temperature as a covariate on the detection parameter with the probability of detecting the geckos active on the building walls increasing with air temperature. This model estimated a mean detection probability of 0.22 (95% CI = 0.14–0.34; estimates at mean value of temp). The effect of year numeric variable (βyear = −0.35; 95% CI = −0.42 to −0.27) highlighted a negative trend in population abundance. Population abundance estimates, obtained from the selected model, were largely in agreement with those obtained by CMR (Fig. 1). In addition, the 95% confidence interval of the annual estimates from CMR and *N*-mixture methods overlapped in all years except 2005, and the mean relative bias (B) between CMR estimates ($\mathrm{CMR}\hat{n}$) and *N*-mixture estimates ($\mathrm{Nmix}\hat{n}$), calculated as $B = (\mathrm{CMR}\hat{n} - \mathrm{Nmix}\hat{n})/\mathrm{CMR}\hat{n}$, was B = 0.27 ± 0.05 (SE). Finally, the temporal trends obtained by both methods were similar, suggesting that the *N*-mixture model with time-for-space substitution was able to capture the long-term dynamics of the gecko population.

4. Discussion

Our results showed how *N*-mixture population estimates were comparable to the values obtained by CMR, and both methods were able to detect long-term population dynamics, specifically highlighting a similar declining trend. Moreover, the values of relative bias observed in our dataset were in line with the expected ones for low detection probability (<0.3) simulation scenarios (Ficetola et al. 2017, Duarte et al. 2018). In general, Duarte et al. (2018) report that *N*-mixture models, in cases of low detection probability and unmodeled heterogeneity in detection, tend to overestimate the real population abundance, whereas Veech et al. (2016) reported that Poisson *N*-mixture models typically underestimate abundance in the presence of intrinsic heterogeneity (i.e., detection probability varies among individuals). In our application, the *N*-mixture model appeared to systematically underestimate population abundance in comparison to CMR; we obtained lower values in comparison with CMR in about 75% of years. Therefore, these findings

seem to be more in line with the simulations of Veech et al. (2016). The overall agreement between N-mixture and CMR estimates let us assume that identifiability problems and other major sources of bias, recently raised against these models (Barker et al. 2017, Link et al. 2018), are not of concern, at least in this study. In the future it would be important to assess the reliability of our *N*-mixture approach in systems with even lower values of detection probability values (i.e., <0.15) that are found when monitoring animals in tropical areas (Ferraz et al. 2011), or snakes (Durso et al. 2011, Steen et al. 2012).

Table 1 Results of model selection of N-mixture models with time-for-space substitution, based on AIC_C. For abbreviations see text.

Model	Parameters	AIC_C	ΔAIC_C	AIC_CWt
λ(year)p(temp)	4	589.33	0.00	0.62
λ(year)p(surv)	4	592.07	2.73	0.16
λ(year)p(.)	3	592.2	2.87	0.15
λ(year)p(rh)	4	595.01	5.68	0.04
λ(year)p(wind)	4	595.36	6.03	0.03

5. Management implications

Many species, of high management and conservation value, have very narrow geographic ranges, few presence locations are known or few populations can be studied. In these situations, the application of CMR protocols to monitor species long-term seems impossible or unsustainable over a prolonged period. The conservation and management of these species may benefit from the application of a more cost-effective monitoring method based on repeated counts of unmarked individuals, instead of a CMR approach. We suggest that wildlife managers interested in long-term population surveys could reduce monitoring costs by using time-for-space substitution, after a period of validation by other independent methods, such as CMR.

Acknowledgments

Thanks are due to the many volunteers (colleagues, students, and friends), that participated to the nights sampling surveys over the years. In some years, the staff of the Province of Genova local police, ARPAL, and of the Association Nuova Acropoli of Genova were present and participated to the

gecko surveys. We are grateful to the Associate Editor, M. J. Mazerolle, and 1 anonymous reviewer for improving our manuscript. The Town of Genova, Italy partially funded this research in 2006. Capture permits were issued by Italian Ministry of Environment (capture permits: SCN/2D/98/8670, SCN/99/2D/12326, SCN/2D/2000/2431, DCN/2D/2002/3026/, DCN/2D/10985/2003/,DPN/IID/2005/6708, DPN/2D/2006/7547, DPN/2007/001058, DPN/2009/0010376, DPN/2010/0010807, 0042466/PNM/2013, 0013862/PNM/2016)

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Chapter 7

Time-for-space substitution in N-mixture models for estimating population trends: a simulation-based evaluation

Part of this chapter is under review as: Costa, A., Salvidio, S., Penner, J., Basile, M. Time-for-space substitution in N-mixture models for estimating population trends: a simulation-based evaluation. *Ecological Modelling*.

Abstract

N-mixture models, that use repeated count data on multiple locations for estimating population abundance, are receiving great interest and their reliability has been evaluated several times. The time-for-space substitution (TSS) in N-mixture models allows to estimate population abundance and trend on a single population, i.e. without spatial replication, for subsequent years. This particular application could be of great interest in ecological studies and conservation programs; however, its reliability has only been evaluated on a single case study so far. In this study we perform a simulation-based evaluation of this particular application of N-mixture model. We generated count data, under 144 simulated scenarios, from a single population surveyed several times per year and subject to different dynamics. We then compared simulated abundance and trend values with TSS estimates. We also evaluated the reliability of a parametric bootstrap goodness-of-fit (GOF) test in predicting the amount of bias in abundance estimate.TSS estimates are overall in good agreement with real abundance. Trend and abundance estimation is mainly affected by detection probability and population size. Bias in trend estimation is also affected by trend direction: being higher in declining populations and particularly in case of low detection probability. The results of GOF test, from our simulation, seem unrelated with the bias observed in abundance estimation, highlighting a low statistical power of this test. After evaluating the reliability of TSS, both against real world data (Costa et al., 2019) and simulations, we suggest that this particular application of N-mixture model could be reliable for monitoring abundance in single populations over several years. Many species have very narrow geographic ranges, or sometimes are known only from few local populations. In these situations, the application of time-consuming protocols, such as CMR seems unrealistic and the conservation and management of these species may benefit from the application of more cost-effective monitoring methods, based on repeated counts of unmarked individuals, such as TSS.

1.Introduction

The study of population abundance and the analysis of its fluctuations over time, i.e. population dynamics, constitutes a fundamental tool in adaptive wildlife monitoring, conservation and management (Williams et al., 2002; Lyndenmayer and Likens, 2009). However, it is often difficult to obtain reliable estimates of population size, or even to accurately assess species presence, given that species within a community and individuals within a population are typically detected imperfectly (MacKenzie et al., 2002; Williams et al., 2002). In order to account for imperfect detection, researchers developed several methods for obtaining reliable estimates of abundance, such as capture-mark-recapture (CMR), removal and distance sampling (Williams et al., 2002; Buckland et al., 2015). These methods rely mostly on individual identification or some sort of auxiliary data (e.g. in distance sampling the perpendicular distance of the observation to the transect) that may be expensive in terms of costs and efforts, impractical to collect or even unsuitable in complex environments or for small and secretive species. Over the last fifteen years the development and application of N-mixture models (Royle, 2004), that allow simultaneous estimation of abundance and detection probability, received large interest from field ecologists (Kéry and Royle, 2016). This method relies on repeated counts of individuals at multiple sites (e.g. line transects or plots) and are highly cost-effective (Ariefiandy et al., 2017; Romano et al., 2017). More recently, some extensions to these models have been formulated, such as removal or double observer data (Chandler et al., 2011), to model open populations in a robust-design fashion (Dail and Madsen, 2010), or even to model species-specific abundance of entire assemblages or communities (e.g. Augustynczik et al. 2019; for a detailed overview see chapter 11 in Kéry and Royle, 2016). Another important feature of N-mixture models is the capability of modelling both the observation process (i.e. detection probability) and the ecological processes (e.g. abundance) as a function of environmental covariates; hence allowing ecological inferences about abundance and density or to address management and conservation actions (e.g., Peterman and Semlitsch, 2013; Balestrieri et al., 2015). Increasing use of N-mixture modelling in monitoring schemes and ecological studies led to a growing interest towards their reliability. Indeed, several studies raised issues with regard to parameter identifiability when model assumptions are violated, or in presence of unmodelled sources of heterogeneity in the abundance or detection processes (Barker et al., 2017; Link et al., 2018). Nevertheless, despite the problems raised, many recent studies

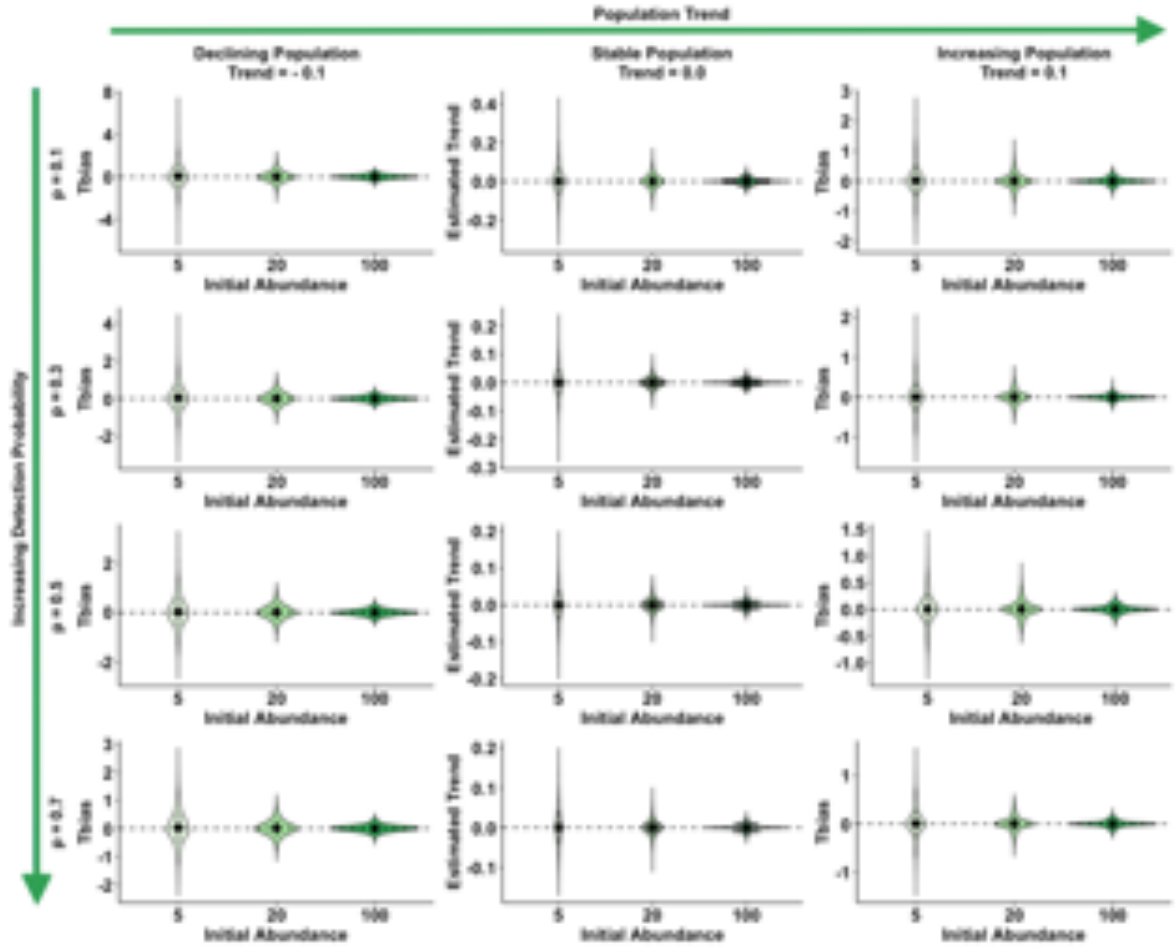

Figure 1 Violin plots of the observed bias in trend estimation (Tbias) for several scenarios, according to different levels of population trend, detection probability, and initial abundance. Black dots and vertical bars inside each violin plot represent the mean and standard deviation, respectively.

confirmed the reliability of this family of models in real-life applications: by comparing them against a hierarchical variant of a capture-recapture model (Kéry, 2018), or by comparing abundance estimates obtained by means of N-mixture models against those estimated with widely accepted methods, such as CMR, removal or distance sampling (Ariefiandy et al., 2014; Priol et al., 2014; Ficetola et al., 2018; Costa et al., 2019). Although N-mixture modelling can be employed in many situations, this approach typically relies on a meta-population design, hence repeated counts of individuals in multiple sampling locations are obtained over time (Kéry and Royle, 2016). However, particularly when dealing with species with a very narrow geographic range and few known populations, a spatially replicated monitoring protocol may impracticable, and only data for single populations in successive years may be available. In these situations, the application of time-for-space substitution (TSS) in the

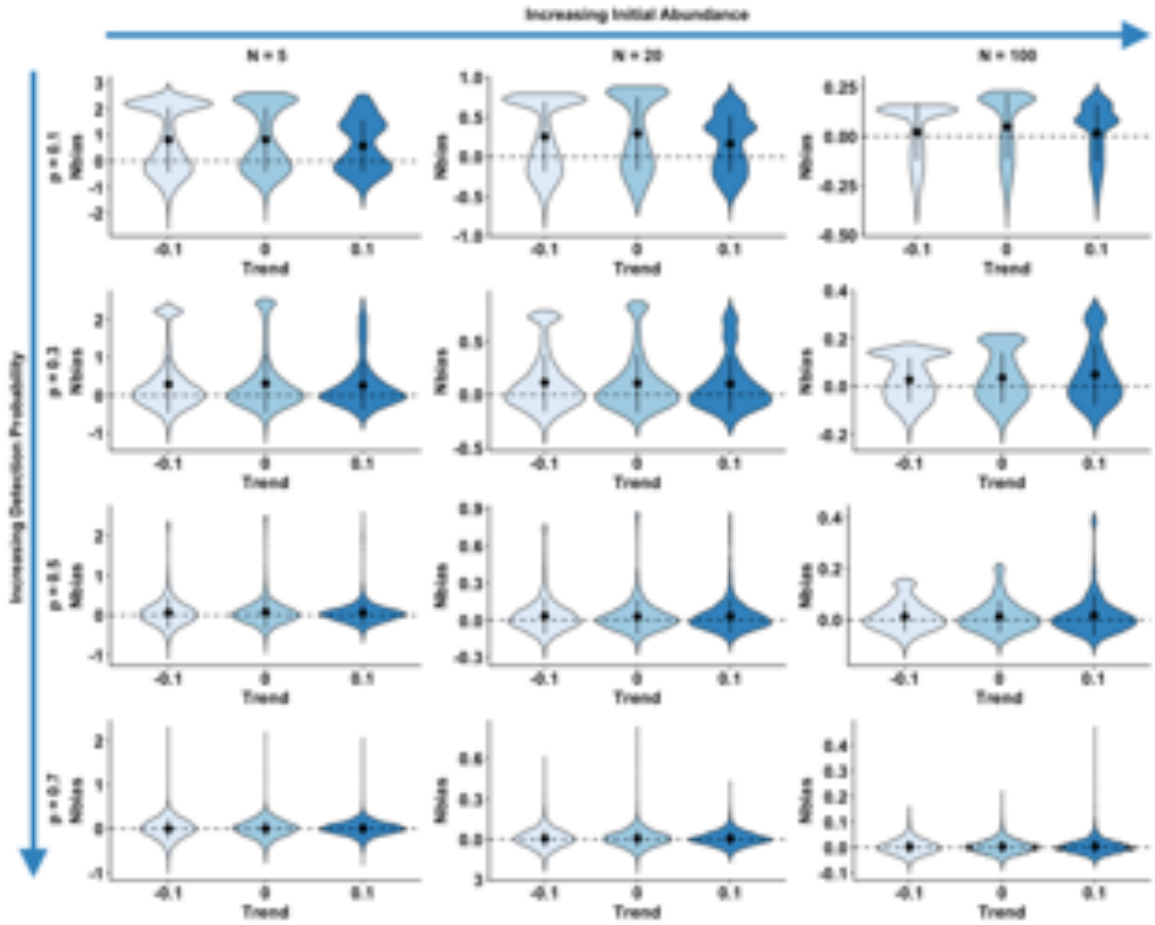

Figure 2 Violin plots of the observed bias in abundance estimation (Nbias) for several scenarios, according to different levels of initial abundance, detection probability and population trend. Black dots and vertical bars inside each violin plot represent the mean and standard deviation, respectively.

N-mixture modelling framework may be a suitable option to monitor a population on a single site over several years (Costa et al., 2019). Time-for-space substitution is applied when multiple counts are conducted over regular time intervals (e.g. months, seasons or years) at the same site, and time replicates (i.e. years) are used in place of space replicates (i.e. sites), while within-year repeated counts (i.e. surveys) are employed as temporal replications, considering the population demographically closed within each year. This framework has been employed by Yamaura et al. (2011) for a multi-species system with detection/non-detection data of bird species obtained during nine consecutive years at a single site in Japan. After Yamaura et al. (2011), TSS has been employed by Costa et al. (2019) on a 20-years spanning dataset of a declining population of an endangered gecko in Italy, finding good agreement with CMR estimates obtained from the same data, and hence validating the TSS framework on a real-life

application. Kéry and Royle (2016) also evaluated this technique against a small simulation scenario, highlighting the need of an in-depth evaluation through a more extensive simulation framework.

Aim of this study is to provide an exhaustive evaluation of the reliability of TSS applied to N-mixture models, and in particular to assess the reliability of abundance and trend estimations over several scenarios, including different population dynamics, initial abundance, detection probability, survey effort or duration of the study, by simulating count data from a single population surveyed several times per year and subject to different dynamics, and then comparing real abundance and trend values with TSS estimates.

2. Materials and methods

2.1 Simulation scenarios

In order to assess the reliability of the TSS in N-mixture model framework we built three main arrays of different scenarios, based on the underlying population dynamics (constant, positive or negative trend), each one containing 48 nested scenarios resulting from combinations of population initial abundance (N = 5, 20, 100; for small, medium and large populations, respectively), study duration (10, 20 years), survey effort (3, 5 surveys/year) and detection probability (p = 0.1, 0.3, 0.5, 0.7), thus obtaining 144 different scenarios. For each of these scenarios, we simulated 1000 datasets obtaining a total of 144000 datasets. Simulated datasets were generated using the code from Kéry and Royle (2016) and a full version of our code is available as supplementary material (Appendix 1). Population dynamics are expressed as a log-linear regression coefficient (Kéry & Royle, 2016): we used a coefficient of T = 0.0 for the 48 constant population models, a coefficient of T = 0.1 for the 48 positive trend scenarios and a coefficient of T = -0.1 for the 48 models accounting for a declining population. Each dataset derived from our simulation process has been analyzed by means of TSS N-mixture models. Our models accounted for Poisson error distribution and the upper boundary (K) was automatically set for each model run; we did not consider negative binomial error distribution since it has been observed to cause infinite or K-truncated abundance estimates (Dennis et al., 2015; Barker et al., 2017). We added a year numeric variable on the abundance side of the formula, in order to model population trend (Kéry and Royle, 2016; Costa et al., 2019) other than initial abundance (Intercept). For each simulated dataset, we stored the real initial abundance (N) and the real trend (T; for those models including population dynamics), along with the estimated abundance (*n*) and trend (*t*) and then we calculated the relative bias for each

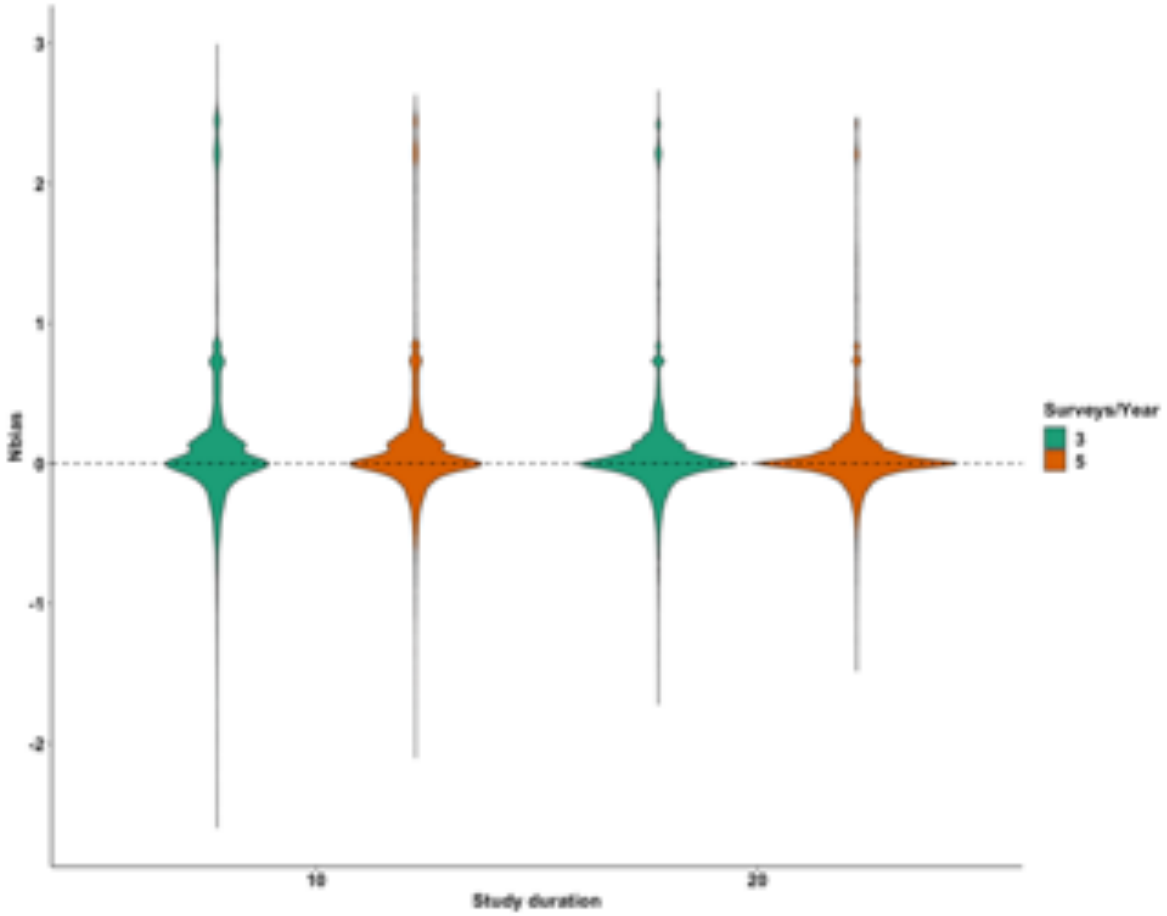

Figure 3 Violin plots of the observed bias in abundance estimation (Nbias) for different levels of study duration (10 and 20 years) and survey effort (3 and 5 surveys per year)

parameter as follows: Nbias = (*n* - N) / N and Tbias = (*t* – T) / T. For constant population models it was not possible to calculate Tbias, because of a division by zero, and therefore the distance of the observed trend from the real one was used. Thus, we obtained a measure of the bias for each simulation, which will assume positive values in case of overestimation of the real parameter or will fall below zero in case of underestimation (Duarte et al., 2018). Moreover, the relative bias can be interpreted as a percent measure of bias: for instance, a bias B*n* = 0.2 means that the given model overestimated abundance by 20%. Considering a value of Nbias < 0.20 (which equals a 20% bias in estimates) as acceptable in monitoring programs, we estimated the threshold values of detection probability needed to achieve this bias value in relation to population sizes, and independently from survey effort or study duration. We calculated the coverage probability of the 95% Confidence Interval for both abundance and trend estimates under a subset of scenarios, and evaluated if scenarios with a 95% or higher coverage rate showed lower bias than those with a lower coverage. Finally, in order to evaluate the effect of unmodelled heterogeneity in abundance on the performance of TSS, we

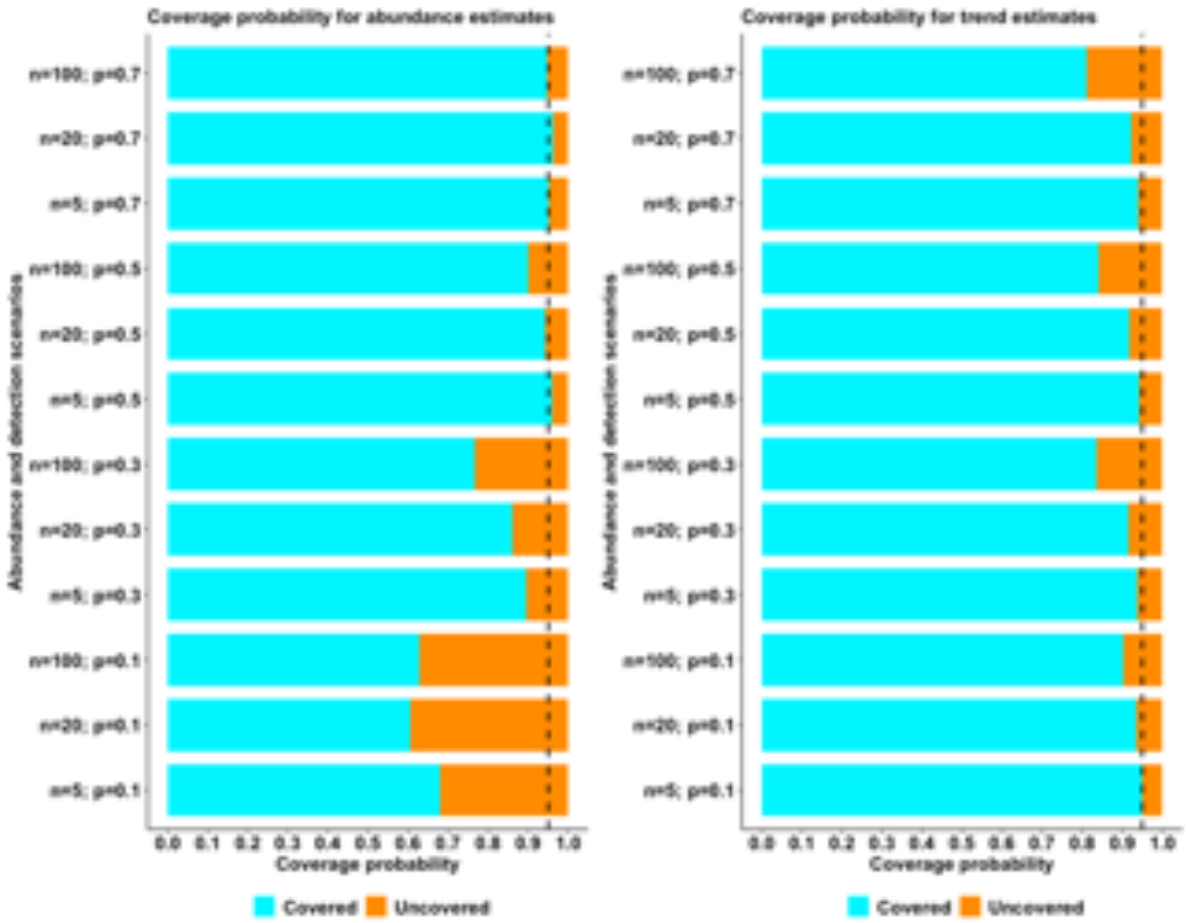

Figure 4 Bar charts representing the proportion of models in which real parameters are covered or not by the 95% confidence interval. The vertical dashed line represents a 0.95 coverage probability.

calculated and retained a measure of heterogeneity for each dataset and compared it with the relative bias in abundance estimation. As a measure of heteroegenity in abundance among years, we adopted the coefficient of variation (CV) of the real abundance of each year of the simulated study (Nhetereogeneity), following the approach proposed by Duarte et al. (2018). We performed this analysis only for the 48 models accounting for a constant population. All analyses were conducted in the R environment with packages "unmarked" (Fiske and Chandler, 2011) and "AHMbook" (Kéry et al., 2016).

2.2 Goodness-of-fit testing

Like any other estimator, also N-mixture models require a set of assumptions (demographic closure within a sampling season and independence of detection, among many – Royle, 2004) to be verified and met. Methods to assess assumption violation in N-mixture models, and hierarchical models in general, are receiving increasing attention and their reliability as good diagnostic tools to assess model adequacy is currently under debate (Warton et al., 2017; Duarte et al., 2018; Knape et al.,

2018). The most adopted method to assess assumption violation in N-mixture models is to employ parametric bootstrapping procedure as a goodness-of-fit (GOF) test (MacKenzie and Bailey, 2004; Kéry and Royle, 2016). Thus, we performed a GOF test on a subset of our simulation scenarios to evaluate the reliability of parametric bootstrap GOF test in identifying bias in N-mixture models with TSS. We simulated again our 48 constant population scenarios, but this time we also performed a Pearson chi-square test using a parametric bootstrap procedure with 1000 resampling, for each fitted model (MacKenzie and Bailey, 2004). We considered models to result in a good fit when the p-value of the Pearson chi-square was > 0.1. Moreover, for each fitted model, we also obtained a measure of overdispersion (c-hat) which assumes that values close to 1.0 are good fitting models, and increase (or decrease towards 0) in case of assumption violations. Then, we compared the relative bias in abundance estimation of each model with its corresponding GOF test results, highlighting whether GOF test is a good predictor of model bias. Since performing parametric bootstrap GOF test is time consuming, for this analysis we reduced the number of simulations for each of the 48 scenarios from 1000 to 200. GOF tests were performed in R environment with package "AICcmodavg" (Mazerolle, 2017).

3. Results

3.1 Simulation scenarios

A graphic summary of results from the simulation scenarios, in trend and abundance estimation, is presented in Figure 1 and 2, respectively, while complete results are reported as supplementary materials (Appendix 2). With regard to trend estimation, as a general rule, bias in estimates is generally higher in declining rather than in stable or increasing populations (Figure 1). Similarly, simulation scenarios accounting for a lower initial abundance (N = 5) experience the higher Tbias while the lower Tbias value is achieved in larger populations (Figure 1). Detection probability seems to have a great influence on trend estimation: the scenarios accounting for a lower detection probability (p = 0.10 and 0.30) being the ones with higher Tbias and, conversely, the ones with higher detectability (p = 0.50 and 0.70) achieving the lower Tbias values. N-mixture models with TSS, within our range of scenarios, seem to systematically overestimate abundance, being the value of Nbias positive in the majority of cases. As already observed for Tbias, also Nbias is higher in populations experiencing a negative trend, rather than population with a positive or constant dynamic (Figure 2). Moreover, bias in abundance is slightly

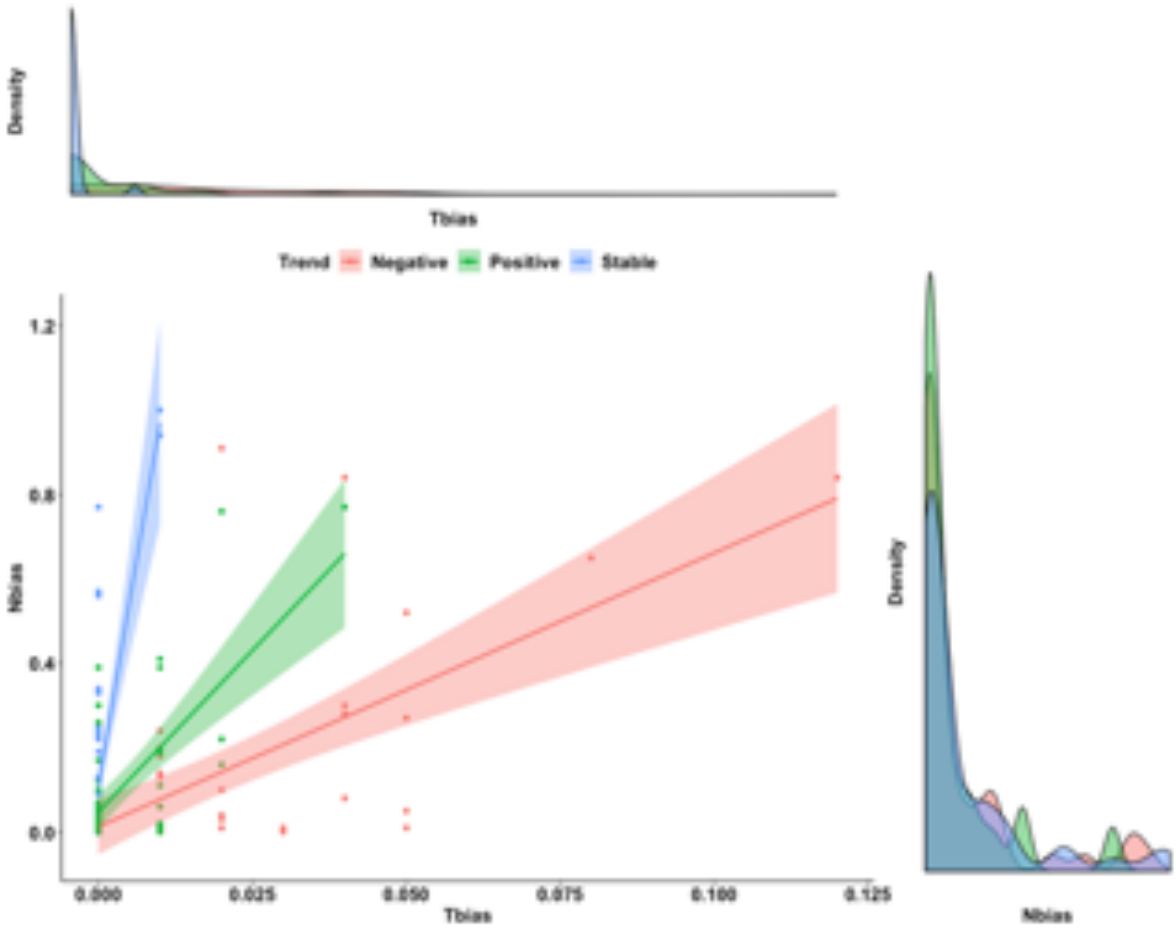

Figure 5 Scatterplots representing the relationship between the absolute value of bias in abundance estimation and the bias in trend estimation, for the 144 simulation scenarios. The density plot on the top and the one on the right represent the distribution of the bias in trend estimation and abundance, respectively.

influenced by initial population size, duration of the study and survey effort (Figure 3). Finally, considering a value of Nbias < 0.20 (which equals a 20% bias in estimates) as acceptable in monitoring programs, with regards to detection probability, the majority of simulations in small population scenarios achieved a Nbias < 0.20 for detection probability $p \geq 0.5$. In medium sized population scenarios, the threshold value of detection probability required to achieve a Nbias < 0.20 decreases to $p \geq 0.3$; while in case of large populations, even the majority of scenarios accounting for a $p \geq 0.1$ show a bias lower that 0.20. With regard to coverage rate of the 95% confidence interval in abundance and trend estimation, the results are summarized in Figure 4. Scenarios characterized by lower detection probability are those experiencing the lower coverage probability for abundance estimates, while the coverage rate for trend estimates is higher also for these scenarios. The mean Nbias of scenarios with a 95% coverage of the real abundance (mean = 0.05; sd = 0.33) is significantly lower than the mean Nbias of uncovered scenarios (mean = 0.54; sd =

0.88; Mann-Whitney test, $p < 0.001$). Also for the bias in trend estimation, the mean bias is lower in scenarios with a coverage rate higher that 0.95 (mean = 0.004; sd = 0.56) than in those with a lower coverage rate (mean = 0.008; sd = 0.28; Mann-Whitney test, $p < 0.001$). For what concerns the relationship between Nbias and Tbias, even when a severe bias in initial abundance is present, the bias in trend estimation remains low, as shown in Figure 5. The observed heterogeneity in abundance (Nhetereogeneity) in the 48 stable population scenarios ranged from 0.03 to 0.95 (mean = 0.25; sd = 0.15). The relationship between heterogeneity in abundance and bias is shown in Figure 6, and the correlation between them is weak but highly significant (Spearman's rho = 0.39; $p < 0.001$).

3.2 *Goodness-of-fit testing*

Results on goodness-of-fit test evaluation on our subset of 48 simulation scenarios, for populations with constant dynamic, are summarized in Figure 7. Overall, the outcome of parametric bootstrap GOF test seems unrelated with the relative bias of abundance estimates. The majority of models evaluated in this simulation passed the threshold p-value ($p = 0.10$) for the GOF test, hence resulting in a theoretical good fit. However, some level of bias was experienced by both models that resulted in a good fit and those that did not pass the GOF test. As shown in Figure 7, a considerable number of models shows a high value of bias, while their GOF p-value > 0.10. At the same time the value of c-hat does not relate with bias. Both regularly, over- and under-dispersed models experience a large range of bias in abundance and trend estimation.

4. Discussion

From our simulation study, we observed how trend and abundance estimation is mainly affected by detection probability and population size. Bias in trend estimation is also affected by trend direction: being higher in declining populations and particularly in case of low detection probability. In these cases, counts become small and may generate unreliable abundance estimates (Royle 2004, McIntyre et al., 2012; Veech et al., 2016; Barker et al., 2017). This pattern in trend estimation has also been observed for the open population N-mixture model (Dail and Madsen, 2011; Ficetola et al., 2018B). At the same time, however, Ficetola et al. (2018B) report that bias in abundance estimation, for the Dail Madsen model, is dependent on detection probability and survey effort, as in the present study, but not on initial population size. In the only evaluation of TSS against real world data

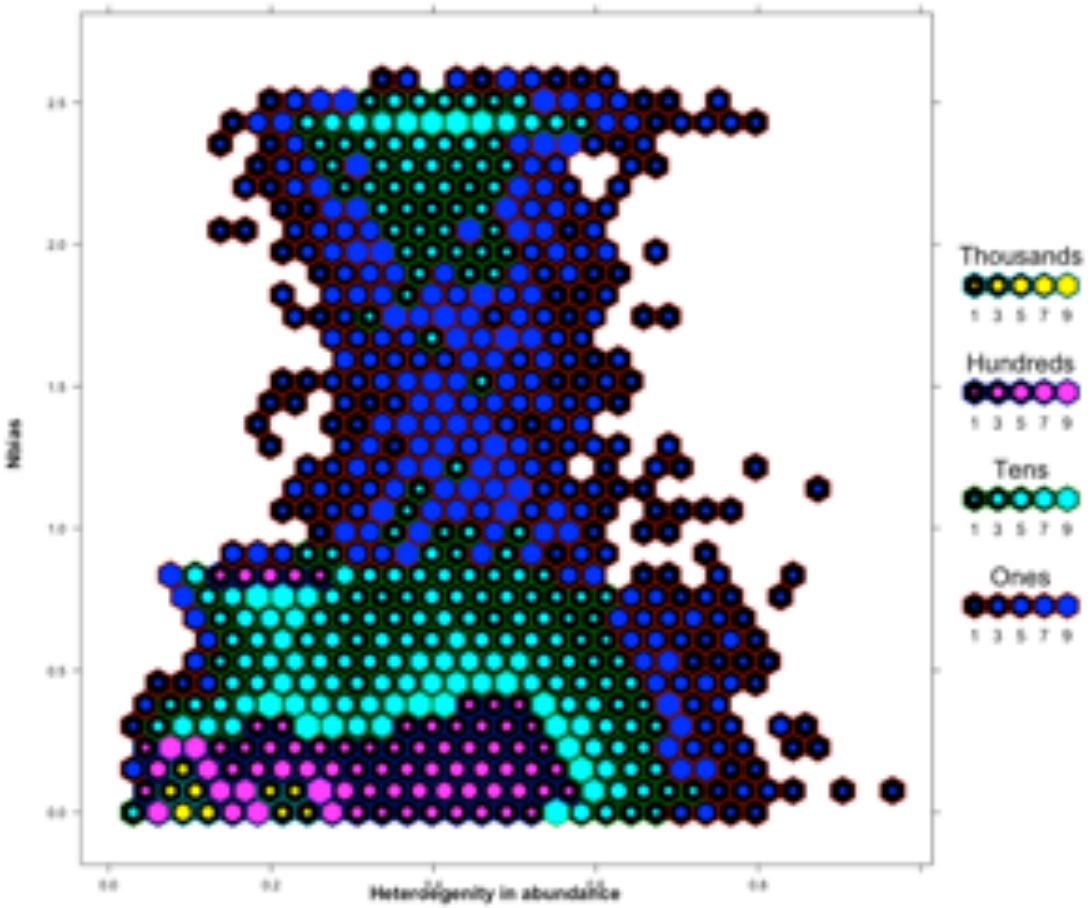

Figure 6 Scatterplot of the relationship between heterogeneity in abundance and the absolute value of bias in abundance estimation. Hexagonal bins represent the number of points falling within each hexagon.

Costa et al. (2019) observed how TSS usually under-estimates abundance; an issue that is known to occur in Poisson N-mixture models in case of intrinsic heterogeneity, due to positive density-dependent detection (Veech et al., 2016). Conversely, in the present study TSS tends to systematically over-estimate abundance: this behavior being known in case of low detection probability or in presence of unmodelled heterogeneity in the detection process (Duarte et al., 2018). Considering only detection probability and population size we observed that at least a detection probability of $p = 0.5$ is required to obtain a bias in abundance estimate $< 20\%$ for small populations, as already suggested by Veech et al (2016) for the classical N-mixture model. In case of larger populations reliable estimates can be obtained for detectability ≥ 0.3 or even ≥ 0.1. Ecologists and wildlife managers usually need to estimate abundance and trend of small populations of rare, cryptic species: in these specific situations, it seems that TSS could not produce reliable estimates of abundance. However, we stress that our scenario for small populations is really severe, indeed in

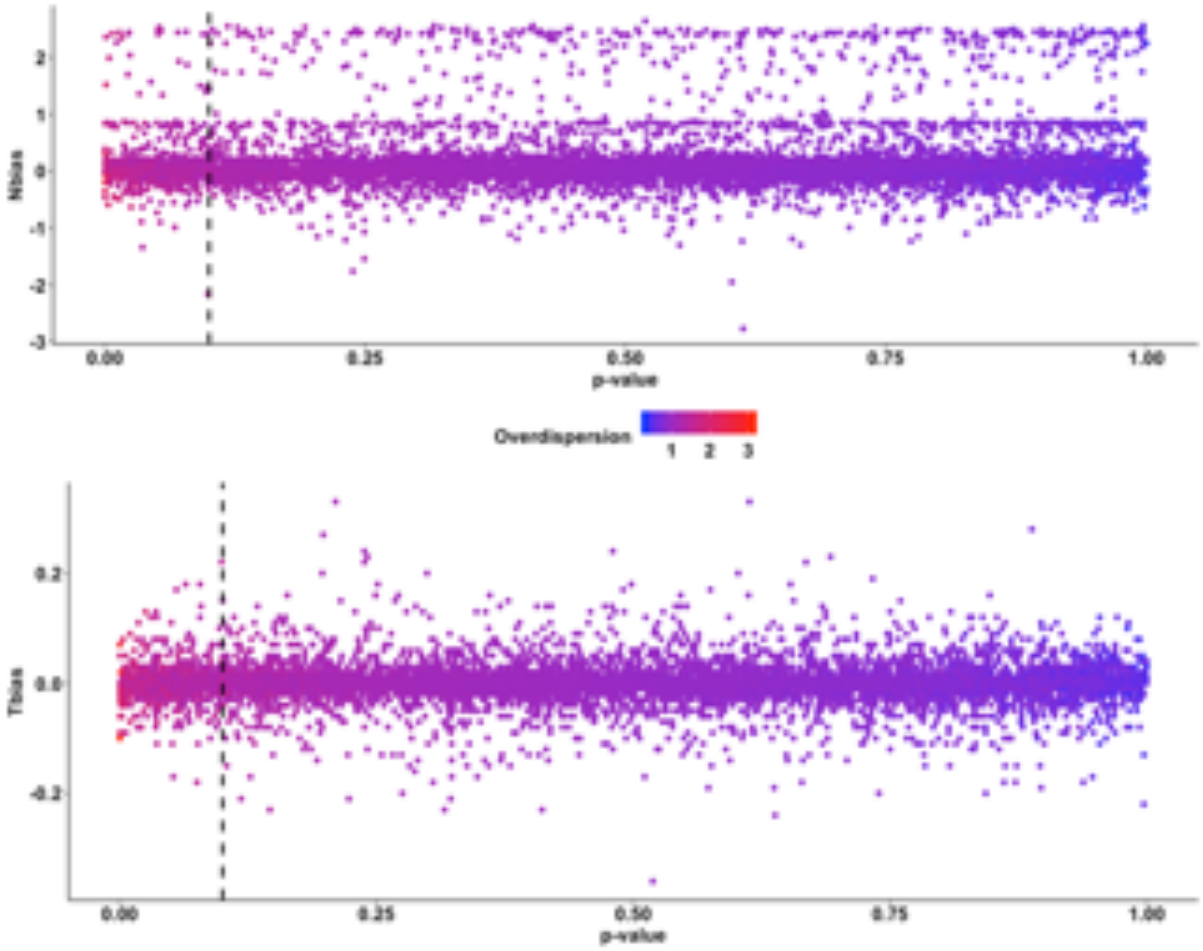

Figure 7 Scatterplots representing the relationship between the significance of the goodness of fit test and the bias in abundance (top) and trend (bottom) estimation. Vertical dashed line represents the significance threshold of the test.

real world application ecologists and managers rarely deal with populations where N = 5, but is rather more likely to deal with larger ones (e.g. Gervasi et al., 2008; Basile et al., 2016). In these cases, an estimation bias lower than 20% is achievable despite low detection probability. Furthermore, results of our study show that, even if a large bias in abundance estimate may occur, associated estimates of population trend are still reliable. One of the major sources of criticism against N-mixture models concerns their lack of robustness in presence of, even slight, assumption violations. Main assumptions of N-mixture models are population closure, independence of detections, absence of individual heterogeneity in detection probability and, lastly, absence of unmodelled heterogeneity in abundance and detection (Kery and Royle, 2016). Among these deviations from model assumptions, those regarding population closure and intrinsic heterogeneity cannot be addressed in N-mixture models, because of the nature of the data used. These latter sources of heterogeneity are those most

investigated by recent studies on the effectiveness of N-mixture models (Veech et al., 2016; Link et al. 2018; Duarte et al., 2018). Unmodelled heterogeneity in abundance and detection, by contrast, can be easily addressed by the use of covariates in N-mixture models, but its effect on model performance has not been tested in depth (Kery and Royle, 2016). We focused on this latter type of heterogeneity in abundance, across sampling years, in the TSS framework. The levels of heterogeneity in abundance in our simulated scenarios were slightly higher than those used in other studies (Duarte et al., 2018), and similar to those observed, for instance, in long-term studies of salamanders (CV = 0.27; Welsh & Conroy, 2001) and geckos (CV = 0.37; Costa et al., 2019) populations. The relationship between Nbias and Nheterogeneity observed in the present study is weak and its effect seems negligible. Moreover, this kind of unmodelled heterogeneity is easily addressable by the inclusion of covariates in the model (Kéry and Royle, 2016). In any case, we suggest that this topic should be analyzed in detail in further studies. N-mixture models in general, like any other method for abundance estimation, require a set of assumptions to be met prior to fitting the model (Royle, 2004; Kéry and Royle, 2016). In our study, the parametric bootstrap GOF test seems to be somewhat unreliable for assessing the fit of TSS. Indeed, both the significance level of the test and its measure of overdispersion were unrelated to the value of the model relative bias. However, Duarte et al. (2018), assessing the reliability of the parametric bootstrap GOF test for N-mixture models, found that the test adequately predicts bias when assumptions are met and detection probability is high, but with low detection probability the amount of false negative results (i.e. GOF test is not significant but estimates are highly biased) increases and the model diagnostic becomes unreliable. From our screening of the parametric bootstrap GOF, it seems that in TSS framework this model diagnostic is even more unreliable. This issue is probably due to the small sample size used in our simulation scenarios (i.e. number of years in TSS or sites in N-mixture model with meta-population design). Even if parametric bootstrap GOF test seems to be unreliable for testing model adequacy, other techniques have been proposed and found to be more reliable in evaluating model fit, such as quasi-coefficient of variation (Duarte et al, 2018) or analysis of residuals (Knape et al., 2018).

Our study represents the first and until now the most complete evaluation of TSS for long-term population monitoring. After evaluating the reliability of TSS, both against real world data (Costa et al., 2019) and against simulations, we suggest that this particular application of N-mixture model

could be reliable for monitoring abundance and estimate trend in single populations, over several years. The poor reliability of the estimates in low detection probability situations, together with small population size, is in our opinion the major flaw of the method. However, a cost-effective and reliable technique allowing abundance or density estimation in these conditions is still lacking, but strongly needed. In more favorable conditions (i.e. $p \geq 0.3$or $N \geq 20$) TSS showed a low bias both in trend and abundance estimation and then we suggest that it should be considered as a good alternative for long term monitoring, after a period of validation with other methods (Lindermayer and Likens, 2010). Our findings are also remarkable in the light of local wildlife regulations or supranational directives, and in particular in the case of the Council of Europe Directive 92/43/EEC, known as Habitat Directive. In fact, article 11 of this Directive requires the application of reliable monitoring protocols in order to estimate the conservation status of dozens of animal and plant species listed in Annex IV. The Directive also imposes to Member States the maintenance of a "favorable" conservation status of the protected species, on the basis of population dynamics and natural range data. However, many of these species have very narrow geographic ranges, or sometimes are known only from few local populations. In these situations, the application of time-consuming protocols, such as CMR, removal or distance sampling, to the species long-term monitoring seems unrealistic and the conservation and management of these species may benefit from the application of more cost-effective monitoring methods based on repeated count of unmarked individuals, such as TSS.

Funding sources

A preliminary idea of this manuscript was conceived and discussed during a stay, funded by the German Academic Exchange Service (DAAD), of AC at the University of Freiburg.

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General synthesis and conclusions

1. Overview of the findings

The present thesis provided a wide range of applications of HMs in herpetology, spanning from the application of Occupancy and N-mixture models for the conservation of salamanders in managed forest, to the evaluation of novel techniques for trend estimation with the use of count data and N-mixture models. In this final section, the main findings of each part of the thesis will be briefly resumed.

2. Part one: conservation of salamanders in managed forests

Among the many causes of the actual amphibian decline, habitat loss and alteration play a key role (Wells, 2007), and forestry is one of the major causes of habitat alteration, fragmentation and loss, both at a global and local scales (Carlson and Groot, 1997; Riffel et al., 2011). Because of that, understanding the possible effects of forestry practices on amphibians' populations is of primary importance, in order to inform forest managers and provide biodiversity-oriented forestry guidelines. Although this obvious need drove many researchers and managers on the study of this topic, there is no general agreement on the effects of forestry practices on amphibians' populations, nor on the possible mitigations to be applied (deManyader and Hunter, 1995). This effect may be due to a variety of causes: i) different levels of forestry and management practices may produce different alterations ii) different species will have different responses to habitat alterations deriving from forestry, and iii) the lack of an adequate method for assessing the effects of forestry may produce spurious results. While the first two causes are predominant and plausible, i.e. every species will respond differently to different levels of habitat disturbance, there is a marked need for reliable methods for assessing the effects of forest management on amphibian populations (see Table 1 in: Otto et al., 2013). In the first part of the present thesis I employed Occupancy and N-mixture models to disclose the ecology of two terrestrial salamanders in managed forests. In the case of chapter one and three, I used two different approaches (Occupancy and N-mixture models) at two different spatial scales, in order to assess the ecological requirements of the Spectacled Salamander, and to drive forest management plans. Chapter one highlights the reliability of Occupancy models for studying the habitat selection of a small terrestrial salamander, on a non-conventional spatial scale, by inferring salamander Occupancy at the level of a single tree. This approach allowed to inform forest managers on which trees should be retained during forestry operations. On chapter three, conversely, I

focused on a larger spatial scale and adopted N-mixture models in order to infer the determinants of salamanders' abundance, rather than occurrence. Chapter three allowed to inform practitioner at a larger spatial scale and provided a sampling framework transposable to other situations. In chapter two, working with a rare species, Occupancy models gave valuable information and helped informing forest managers. In particular Occupancy models allowed to disclose the importance of Fine Woody Debris (FWD) for the conservation of the Golden Alpine Salamander. FWD indeed, plays a key role in providing suitable habitat for this endangered amphibian. The ecological importance of FWD is generally poorly studied, probably underestimated (Goszczyński et al., 2007; Indermaur and Schmidt, 2011), and in the present case has been disclosed thanks to the use of Occupancy modelling. In conclusion, the application of HMs to forest amphibians in general, and salamanders in particular, gave reliable results and information, valuable to inform forest managers in order to take into account biodiversity conservation during forestry operations.

3. Part two: N-mixture models in amphibian population monitoring

Information on population size are important to inform conservation and management of animal populations. Usually, capture-mark-recapture (CMR) is considered the most robust technique to estimate population abundance, although its expensiveness of application. N-mixture models are an ideal alternative to CMR or removal sampling for population monitoring. However, N-mixture models have some limitations, and their reliability has been criticized. In particular, the reliability of N-mixture models has been questioned, mainly because parameter identification issues in case of assumption violations and unmodelled heterogeneity on the detection or abundance processes (Barker et al., 2017; Link et al., 2018). Despite many authors focused on the reliability of these models, and validated them against common techniques for abundance estimation (Ariefiandy et al., 2014; Priol et al., 2014; Ficetola et al., 2018; Kery, 2018; Costa et al., 2019), some skepticism still remains. Accordingly, increasing the amount of real world applications of N-mixture models, and evaluating their reliability with external methods for abundance estimation, is of primary importance and encouraged (Bötsch et al., 2019). In this context, in chapter four and five, I presented the application of N-mixture models under two different situations. In chapter four, Dail and Madsen (2010) N-mixture model has been applied to call counts of the Stripeless Tree-Frog (*Hyla meridionalis*). In this situation, considering

the difficulty of capturing and marking tree-frogs, and the logistical difficulties of accessing sampling locations, using call counts could gave great advantages for monitoring this species. In chapter five, I focused on multinomial N-mixture models, based on multiple observer protocols, that can be considered as hierarchical extensions of simple CMR. These models are usually applied in estimating abundance of animals with large body size and high detection probabilities. In this chapter, by contrast, I applied and evaluated the reliability of multinomial N-Mixture models with small and cryptic terrestrial salamanders, found in different environment, where populations possess different levels of detectability and density. The reliability of multinomial N-mixture models has been assessed: i) by comparing abundance estimates with those obtained with other reference methods under different environmental conditions, ii) by a simulation study, and iii) by evaluating the effect of heterogeneity in the data on the precision of the estimates. Results from this study confirmed that these models are highly reliable for estimating abundance (Kery, 2018). Moreover, I highlighted how moderate levels of unmodelled heterogeneity in abundance, which is a known source of bias in binomial N-mixture models (Kery and Royle, 2016), does not affect precision of abundance and detection estimates in multinomial N-mixture models. Furthermore, since the application of the multiple observer protocol does not require multiple visits, these models are even more cost-effective than binomial N-mixture models, based on count data. The results obtained in chapter five increase the trust on the reliability of these models to conditions of low abundance and detection probability. For these reasons, the use of multinomial N-mixture models is encouraged in the monitoring of amphibian populations.

4. Part three: Time-for-space substitution in N-mixture model for long term monitoring

N-mixture models are typically used for repeated surveys at multiple sites (Kéry and Royle, 2016), i.e. employing a meta-population design, but sometimes only isolated populations are available and spatial replication is not an option. In these cases, only monitoring data for single sites, or populations, obtained in successive years are available, and the analysis of such data with typical N-mixture models is not possible. However, in this case, is still possible to analyze count data with N-mixture models, by applying a Time-for-space substitution (TSS). TSS in *N*-mixture models consists of substituting space replicates (i.e., sites) by time replicates (i.e., years), and within-year repeated counts (i.e., surveys) are employed as temporal replications. This framework

has been employed by Yamaura et al. (2011), but without a formal validation and evaluation of its reliability. In chapter six and seven, I focused on the application of TSS in order to assess its reliability for long term monitoring of animal populations, without the need of spatial replication. In chapter six, I found good agreement between abundance estimates obtained with TSS and CMR, for a twenty years long data-set of a European leaf-toed gecko population. TSS also reliably identified the declining trend of the study population. In chapter seven, I extended the evaluation of this modelling framework, by testing the reliability of TSS on a large simulation framework. I found that, also for low values of detection probability and abundance, TSS can give reliable estimates of abundance and trend, achieving a bias lower than 10% of the estimate. This work represents, to my knowledge, the first in-depth evaluation of TSS. In the light of these findings, considering that many species have very narrow geographic ranges, few presence locations are known or few populations can be studied, TSS represent a valuable alternative to other techniques for abundance estimation.

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Supplementary Materials

SUPPLEMENTARY MATERIALS TO:

Chapter 4: Estimating abundance of the Stripeless tree-frog Hyla meridionalis by means of replicated call counts

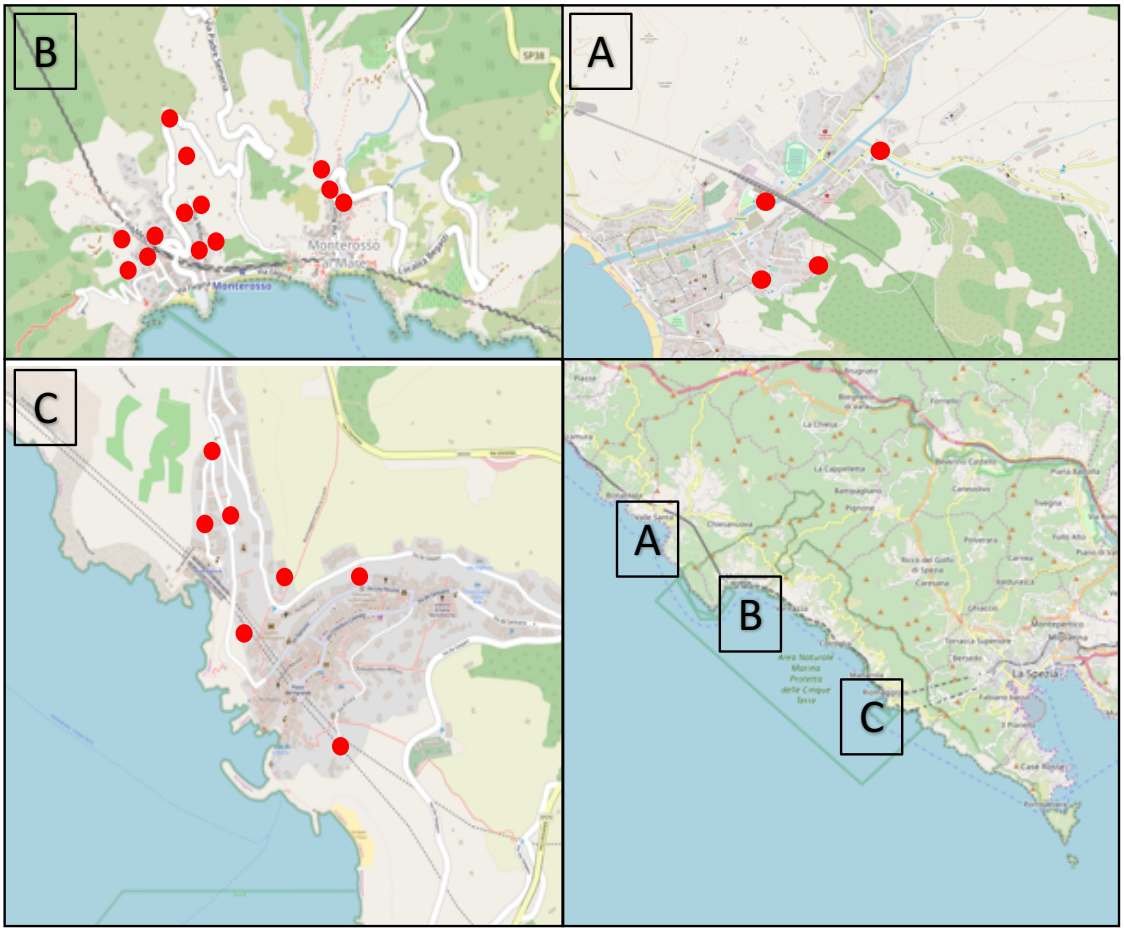


Fig. A1. Map of the sampling sites in the Cinque Terre National Park (lower right corner): A) Levanto; B) Monterosso; C) Riomaggiore. Maps for the current figure are distributed under an Open Data Commons/Open Database License by the ©OpenStreetMap contributors.

Table A1. Counts of males at each site for 2017 and 2018 surveys

site/survey	2017.1	2017.2	2017.3	2018.1	2018.2	2018.3	2018.4
1	NA	NA	NA	1	1	0	0
2	0	3	2	2	3	3	4
3	1	6	4	2	3	3	4
4	0	0	1	0	0	0	0
5	5	4	0	1	3	3	3
6	5	4	4	NA	NA	NA	NA
7	0	0	0	0	0	0	0
8	4	3	2	1	2	2	1
9	2	3	4	1	1	1	0
10	2	4	4	4	3	3	2
11	4	1	1	3	3	2	0
12	4	1	2	2	1	0	1
13	1	0	0	0	0	0	0
14	4	3	4	3	2	2	3
15	1	2	1	0	0	2	0
16	5	3	4	4	5	4	2
17	2	1	2	0	1	0	0
18	1	1	2	3	3	3	2
19	0	0	1	2	2	0	0
20	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0
22	0	0	1	0	0	1	1
23	1	3	1	1	1	1	1
24	2	3	2	3	3	2	3

SUPPLEMENTARY MATERIALS TO:

Chapter 5: Reliability of multinomial N-Mixture models for estimating abundance of small terrestrial vertebrates

Appendix 1. Includes one function, two tables and one figure

Function 1. "Unmarked" custom function (PiFun) to be used in "gmultimix" and "multinomPois" models for a triple independent observer protocol, with seven possible outcomes. Under this sampling protocol an individual could be detected: by observer A only (100), by B only (010), by C only (001), by A and B but not C (110), by A and C but not B (101), by B and C but not A (011), by all three A, B and C (111).

```
triplePiFun<-function (p)
{
  M <- nrow(p)
  pi <- matrix(NA, M, 7)
  pi[, 1] <- p[, 1] * (1 - p[, 2])*(1 - p[, 3])
  pi[, 2] <- p[, 2] * (1 - p[, 1])*(1 - p[, 3])
  pi[, 3] <- p[, 3] * (1 - p[, 1])*(1 - p[, 2])
  pi[, 4] <- p[, 1] * p[, 2]*(1 - p[, 3])
  pi[, 5] <- p[, 1] * p[, 3]*(1 - p[, 2])
  pi[, 6] <- p[, 2] * p[, 3]*(1 - p[, 1])
  pi[, 7] <- p[, 1] * p[, 2]* p[, 3]
  return(pi)
}
```

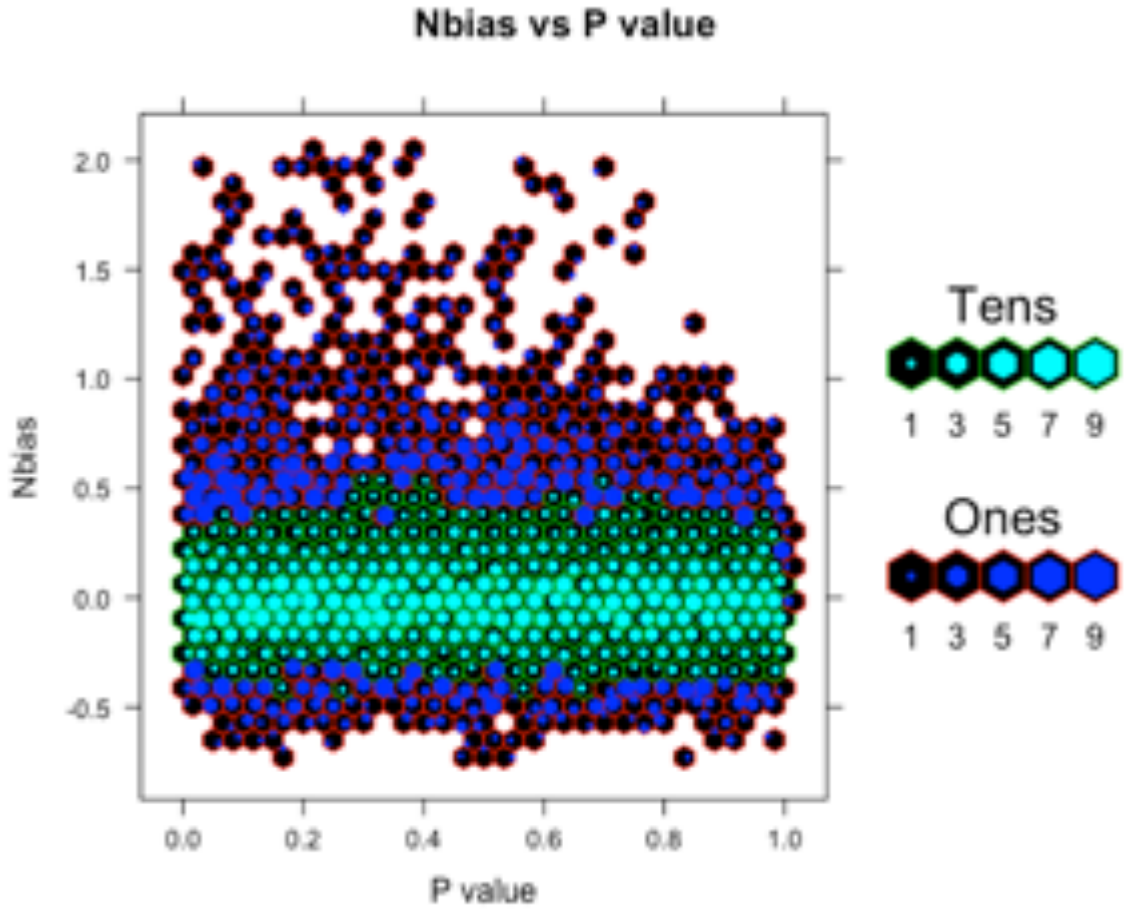
Table 1. Results of model selection for Cave 1, Cave 2 and Woodland, according to AICc. Mod.Avg. column indicates whether model averaged predictions have been used or not.

Subterranean environment	**Cave 1**			
	Model	AICc	ΔAICc	Mod. Avg.
	λ(.) p(observer)	201.61	-	No
	λ(.) p(.)	204.85	3.24	
	Cave 2			
	Model	AICc	ΔAICc	Mod. Avg.
	λ(.) p(observer)	-94.99	-	No
	λ (.) p(.)	-97.12	2.13	
Woodland environment	**Three Observers A+B+C**			
	Model	AICc	ΔAICc	Mod. Avg.
	λ (.) p(.)	156.28	-	No
	λ(.) p(observer)	160.68	4.4	
	Observers A+B			
	Model	AICc	ΔAICc	Mod. Avg.
	λ (.) p(.)	99.10	-	Yes
	λ(.) p(observer)	100.69	1.59	
	Observers A+C			
	Model	AICc	ΔAICc	Mod. Avg.
	λ (.) p(.)	105.40	-	No
	λ(.) p(observer)	107.95	2.55	
	Observers B+C			
	Model	AICc	ΔAICc	Mod. Avg.
	λ (.) p(.)	94.69	-	Yes
	λ(.) p(observer)	96.59	1.90	

Table 2. Pearson's product-moment correlation (correlation coefficient and *p*-value) between *Nbias* and *Nheterogenity* for all double observer simulation scenarios.

Scenario	Pearson's Correlation Coefficient	*p*-value
λ = 3; p = 0.2	0.04	0.36
λ = 3; p = 0.3	-0.02	0.88
λ = 3; p = 0.4	0.00	0.95
λ = 10; p = 0.2	-0.01	0.48
λ = 10; p = 0.3	0.00	0.61
λ = 10; p = 0.4	-0.01	0.30

Figure 1. Scatterplot representing the relationship between the *p*-value of the parametric bootstrap goodness of fit test and *Nbias*. Hexagonal bins represent the number of points falling within the hexagon surface.



SUPPLEMENTARY MATERIALS TO:

Chapter 7: Time-for-space substitution in N-mixture models for estimating population trends: a simulation-based evaluation

Appendix 1. Example of the code used for data simulation, model fitting and results storage

```
#open packages for the simulation
library("AHMbook")
library("AICcmodavg")
library("unmarked")

#Example of a positive trend scenario
#n years=10
#n survey/year=3
#N=5
#trend=0.0
#p=0.1
simrep <- 1000 # Number of simreps
resultsPT1 <- array(NA, dim = c(simrep, 18)) # Array for results
for(i in 1:simrep){
  cat("Simrep", i, "\n")
  data<-simpleNmix(nyear = 10, nrep = 3, beta0 = 1.61, beta1 = 0.1, alpha0 = -2.197,
 alpha1 = 0,alpha2 = 0, show.plot = FALSE) # Simulate a data set
  umf <- unmarkedFramePCount(y = data$C, siteCovs = data.frame(Time = data$Time),
  obsCov = NULL) # Unmarked frame
  Kset<-(max(data$C))*10+300 # Upper integration limit for Lambda
  tryCatch(fm1 <- pcount(~1 ~Time, data = umf,K=Kset, mixture="P"),error=function(e){})
  CI<-confint(fm1, type="state", level = 0.95)
  resultsPT1[i, 1:3] <- round(coef(fm1),2)
  resultsPT1[i, 4] <- round(qlogis(data$p[1,1]),2)
  resultsPT1[i, 5] <- round(data$beta0,2)
  resultsPT1[i, 6] <- round(data$beta1,2)
  resultsPT1[i, 7] <-round(((coef(fm1)[2])-(data$beta1))/(data$beta1),2)
  resultsPT1[i, 8] <-round(((coef(fm1)[1])-(data$beta0))/(data$beta0),2)
  resultsPT1[i, 9] <-round((sd(data$N))/(mean(data$N)),2)
  resultsPT1[i, 10] <-round((sd(data$C))/(mean(data$C)),2)
  resultsPT1[i, 11] <-round(CI[1,1],2)
  resultsPT1[i, 12] <-round(CI[1,2],2)
  resultsPT1[i, 13] <-round(CI[2,1],2)
  resultsPT1[i, 14] <-round(CI[2,2],2)
  resultsPT1[i, 15] <-findInterval(round(data$beta0,2),
 c((round(CI[1,1],2)),(round(CI[1,2],2))))
  resultsPT1[i, 16] <-findInterval(round(data$beta1,2),
 c((round(CI[2,1],2)),(round(CI[2,2],2))))
  resultsPT1[i, 17] <-data$nyear
  resultsPT1[i, 18] <-data$nrep
}
colnames(resultsPT1) <-
 c("lam(Int)","lam(Time)","p(Int)","Preal","Nreal","Treal","Tbias","Nbias","Nheterogeneit
 y","Pheterogeneity","lowN","upN","lowT","upT","CoverageN","CoverageT","Nyear","Nrep")
```

```
#Example including GOF testing
#n years=10
#n survey/year=3
#N=5
#trend=0.0
#p=0.1
simrep <- 200 # Number of simreps
resultsGOF1 <- array(NA, dim = c(simrep, 20)) # Array for resultsGOF
for(i in 1:simrep){
  cat("Simrep", i, "\n")
  data<-simpleNmix(nyear = 10, nrep = 3, beta0 = 1.61, beta1 = 0.0000001, alpha0 = -
2.197, alpha1 = 0,alpha2 = 0, show.plot = FALSE) # Simulate a data set
  umf <- unmarkedFramePCount(y = data$C, siteCovs = data.frame(Time = data$Time),
  obsCov = NULL)
  tryCatch(fm1 <- pcount(~1 ~Time, data = umf,K=300, mixture="P"),error=function(e){})
  CI<-confint(fm1, type="state", level = 0.95)
  tryCatch(gof<- Nmix.gof.test(fm1, nsim = 1000, plot.hist = FALSE, report =
NULL),error=function(e){})
  resultsGOF1[i, 1:3] <- round(coef(fm1),2)
  resultsGOF1[i, 4] <- round(qlogis(data$p[1,1]),2)
  resultsGOF1[i, 5] <- round(data$beta0,2)
  resultsGOF1[i, 6] <- round(data$beta1,2)
  resultsGOF1[i, 7] <-round(((coef(fm1)[2])-(data$beta1)),2)
  resultsGOF1[i, 8] <-round(((coef(fm1)[1])-(data$beta0))/(data$beta0),2)
  resultsGOF1[i, 9] <-round((sd(data$N))/(mean(data$N)),2)
  resultsGOF1[i, 10] <-round((sd(data$C))/(mean(data$C)),2)
  resultsGOF1[i, 11] <-round(CI[1,1],2)
  resultsGOF1[i, 12] <-round(CI[1,2],2)
  resultsGOF1[i, 13] <-round(CI[2,1],2)
  resultsGOF1[i, 14] <-round(CI[2,2],2)
  resultsGOF1[i, 15] <-findInterval(round(data$beta0,2),
c((round(CI[1,1],2)),(round(CI[1,2],2))))
  resultsGOF1[i, 16] <-findInterval(round(data$beta1,2),
c((round(CI[2,1],2)),(round(CI[2,2],2))))
  resultsGOF1[i, 17] <-data$nyear
  resultsGOF1[i, 18] <-data$nrep
  resultsGOF1[i, 19] <-gof$p.value
  resultsGOF1[i, 20] <-round(gof$c.hat.est,2)
}
colnames(resultsGOF1) <-
c("lam(Int)","lam(Time)","p(Int)","Preal","Nreal","Treal","Tbias","Nbias","Nheterogeneit
y","Pheterogeneity","lowN","upN","lowT","upT","CoverageN","CoverageT","Nyear","Nrep","go
f.p","gof.chat")
```

Appendix 2. Detailed results of simulations

Table 1 Mean and Standard Deviation of Nbias

			Negative Trend						Stable						Positive Trend					
			N = 5		N = 20		N = 100		N = 5		N = 20		N = 100		N = 5		N = 20		N = 100	
			Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
10 Years	3 Surveys / Year	p = 0.1	0,91	1,51	0,28	0,49	0,01	0,17	1,00	1,34	0,33	0,51	0,05	0,19	0,76	1,22	0,22	0,44	0,02	0,18
		p = 0.3	0,52	1,00	0,18	0,34	0,03	0,11	0,56	1,02	0,22	0,38	0,04	0,13	0,39	0,84	0,16	0,33	0,07	0,17
		p = 0.5	0,14	0,56	0,06	0,21	0,02	0,07	0,19	0,59	0,06	0,22	0,02	0,08	0,17	0,52	0,06	0,20	0,03	0,11
		p = 0,7	0,01	0,29	0,01	0,08	0,01	0,04	0,02	0,24	0,01	0,10	0,00	0,04	0,02	0,24	0,01	0,07	0,01	0,05
	5 Surveys / Year	p = 0.1	0,84	1,25	0,30	0,43	0,03	0,14	0,94	1,24	0,34	0,47	0,06	0,16	0,77	1,07	0,26	0,38	0,06	0,15
		p = 0.3	0,28	0,72	0,13	0,28	0,03	0,09	0,33	0,79	0,13	0,29	0,04	0,11	0,30	0,67	0,12	0,28	0,07	0,14
		p = 0.5	0,05	0,37	0,04	0,15	0,01	0,06	0,05	0,36	0,02	0,13	0,02	0,06	0,05	0,31	0,03	0,13	0,02	0,07
		p = 0,7	-0,01	0,25	0,00	0,07	0,00	0,03	0,00	0,20	0,00	0,06	0,00	0,03	0,00	0,17	0,00	0,06	0,00	0,03
20 Years	3 Surveys / Year	p = 0.1	0,84	1,26	0,24	0,45	0,02	0,15	0,77	1,23	0,25	0,46	0,04	0,16	0,41	0,82	0,07	0,29	-0,02	0,12
		p = 0.3	0,27	0,70	0,10	0,26	0,02	0,09	0,24	0,67	0,09	0,26	0,03	0,10	0,19	0,51	0,07	0,21	0,04	0,12
		p = 0.5	0,03	0,31	0,02	0,11	0,01	0,05	0,06	0,31	0,02	0,11	0,01	0,06	0,04	0,23	0,02	0,10	0,01	0,06
		p = 0,7	0,00	0,19	0,00	0,06	0,00	0,03	-0,03	0,14	0,01	0,05	0,00	0,02	0,01	0,12	0,00	0,04	0,00	0,02
	5 Surveys / Year	p = 0.1	0,65	1,09	0,20	0,38	0,02	0,12	0,57	1,04	0,23	0,40	0,05	0,14	0,39	0,71	0,10	0,25	0,00	0,10
		p = 0.3	0,08	0,37	0,05	0,17	0,02	0,07	0,10	0,38	0,05	0,17	0,03	0,08	0,11	0,33	0,05	0,15	0,03	0,09
		p = 0.5	0,01	0,20	0,01	0,07	0,01	0,04	0,01	0,16	0,01	0,07	0,01	0,04	0,02	0,14	0,01	0,07	0,01	0,04
		p = 0,7	-0,01	0,17	0,00	0,05	0,00	0,02	-0,01	0,14	0,00	0,04	0,00	0,02	0,00	0,11	0,00	0,03	0,00	0,02

Table 2 Mean and Standard Deviation of Tbias

			Negative Trend						Stable						Positive Trend					
			N = 5		N = 20		N = 100		N = 5		N = 20		N = 100		N = 5		N = 20		N = 100	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 Years	3 Surveys / Year	p = 0.1	0,02	2,07	0,04	0,73	0,01	0,29	-0,01	0,11	0,00	0,05	0,00	0,02	0,02	0,77	0,02	0,38	0,00	0,17
		p = 0.3	0,05	0,95	0,01	0,44	0,00	0,19	0,00	0,07	0,00	0,03	0,00	0,02	0,00	0,50	0,02	0,24	0,00	0,11
		p = 0.5	0,01	0,79	0,01	0,37	0,00	0,17	0,00	0,06	0,00	0,03	0,00	0,01	0,00	0,41	0,00	0,22	0,00	0,10
		p = 0,7	0,05	0,72	0,03	0,35	0,00	0,16	0,00	0,05	0,00	0,03	0,00	0,01	0,01	0,42	0,01	0,21	0,00	0,09
	5 Surveys / Year	p = 0.1	0,04	1,24	0,04	0,56	0,02	0,25	0,01	0,09	0,00	0,04	0,00	0,02	0,04	0,67	0,00	0,32	-0,01	0,14
		p = 0.3	0,04	0,79	0,01	0,40	0,00	0,18	0,00	0,06	0,00	0,03	0,00	0,01	0,00	0,46	0,00	0,24	0,00	0,10
		p = 0.5	0,05	0,75	0,02	0,36	0,01	0,16	0,00	0,05	0,00	0,03	0,00	0,01	0,00	0,40	0,00	0,21	0,00	0,09
		p = 0,7	0,02	0,70	0,01	0,34	0,00	0,16	0,00	0,05	0,00	0,03	0,00	0,01	0,01	0,38	0,00	0,19	0,00	0,09
20 Years	3 Surveys / Year	p = 0.1	0,12	0,75	0,01	0,31	0,01	0,13	0,00	0,04	0,00	0,02	0,00	0,01	0,01	0,21	0,00	0,10	0,00	0,04
		p = 0.3	0,05	0,41	0,02	0,21	0,00	0,09	0,00	0,02	0,00	0,01	0,00	0,01	0,01	0,14	0,00	0,07	0,00	0,03
		p = 0.5	0,00	0,35	0,00	0,17	0,00	0,08	0,00	0,02	0,00	0,01	0,00	0,01	0,00	0,12	0,00	0,06	0,00	0,03
		p = 0,7	0,03	0,35	0,00	0,15	0,00	0,07	0,00	0,02	0,00	0,01	0,00	0,00	0,00	0,11	0,00	0,06	0,00	0,02
	5 Surveys / Year	p = 0.1	0,08	0,55	0,01	0,26	0,00	0,11	0,00	0,03	0,00	0,01	0,00	0,01	0,01	0,18	0,00	0,09	0,00	0,04
		p = 0.3	0,04	0,36	0,00	0,19	0,00	0,08	0,00	0,02	0,00	0,01	0,00	0,01	0,01	0,12	0,00	0,06	0,00	0,03
		p = 0.5	0,02	0,31	0,01	0,17	0,00	0,07	0,00	0,02	0,00	0,01	0,00	0,00	0,01	0,12	0,00	0,06	0,00	0,03
		p = 0,7	0,01	0,31	0,01	0,15	0,00	0,07	0,00	0,02	0,00	0,01	0,00	0,00	0,00	0,11	0,00	0,05	0,00	0,02